STUDIES IN RENAL PATHOLOGY

by

MOHAMMAD SADEK SABOUR
M.B.,B.Ch.,D.M., M.R.C.P.Ed., M.R.C.P.

Lecturer, Department of Internal Medicine and physician, The Renal Clinic, Ain-Shams University, Cairo, U.A.R.

Thesis submitted for the degree of Doctor of Philosophy.

Department of Pathology,
UNIVERSITY OF EDINBURGH.

1962.

INDEX.

ACKNOWLEDGEMENTS.

The work presented in this thesis has been undertaken in the Department of Pathology, University of Edinburgh. I express with pleasure my indebtedness to Professor G.L. Montgomery, with whose Department I have been happily associated as a research worker for the last two years. His continuous encouragement and his great interest in the work I have been doing, as well as his advice and support, have had the utmost value in enabling me to carry out this work.

I must record my special gratitude to Dr. Mary K. MacDonald, who taught me the techniques of tissue preparation and embedding, ultra-thin section cutting and the use of the electron microscope. She made familiar to me the language of renal ultrastructure and her co-operation and interpretation of the results in all the studies presented in this thesis was extremely helpful.

The chapters on the study of human diabetic renal biopsies, the mechanisms of concentration and dilution of the urine and the nephropathy of potassium depletion were carried out in collaboration with Dr. J.S. Robson and Dr. A.T. Lambie, of the Department of Therapeutics. I am very grateful to both of them and especially to Dr. Robson for his views, and suggestions in various aspects of renal physiology and to Dr. Lambie for the chemical data on the potassium deficient rats.

The prednisolone-nephropathy experiment was done in collaboration with Dr. Ogilvie, to whom I am most grateful for the opportunity of studying this valuable tissue and for his very careful study of the light

microscopy in these animals.

The magnesium-deficiency experiment was done in collaboration with Dr. S. Hanna in the Department of Chemical Pathology, Postgraduate School of Medicine, London. His expert work in carrying out the chemical results presented as well as his valuable suggestions were of great help.

Finally, I should like to express by thanks to Mr. G. Wilson, Miss M. Combe and to the Department of Microphotography, University of Edinburgh and to its director, Mr. T.C. Dodds, for the vast amount of work they had to do in association with this research, and to the Senatus Academicus of the University of Edinburgh for granting me a Senior Research Fellowship.

FOREWARD.

The history of medical and biologic science makes it clear that major advances in basic problems of structure and function stem largely from use of appropriate new techniques and ideas. Even the brilliant Ludwig (81) could not formulate his important theory of passive ultrafiltration of blood plasma until Bowman had made his amazingly detailed, accurate study of the Malpighian corpuscle (15). While Bowman, in turn, by the use of the greatly improved techniques and microscopes of the nineteenth century was enabled to see much more than the seventeenth century master, Malpighi.

Today's phsyical science and technology have produced an entirely new microscope, the electron microscope. With the aid of this instrument, a further chapter in biological history is now being written, that of the "fine structure" or ultrastructure" of tissue (62). A coarse probe cannot be used to search out a fine crevice. Light is the probe that is employed in the ordinary microscope, and the coarseness of this probe is unalterably set by the wave length of the visible light (0.4 - 0.7 μ). No matter what lens system is employed, light cannot be used to distinguish clearly the structure of objects that are smaller and no farther apart than about half its wave length (about 0.2 μ). Since the unaided eye can distinguish fine lines that are about 0.2 mm. apart, there is not much point in using a magnification of more than 1,000 times in the light microscope because the human eye at that magnification can distinguish anything that is revealed by light at that wave length. Greater magnifications than 1,000 times yield a larger picture but reveal no further detail.

The revolutionary development in the microscope, which increased its resolving power a hundred or more times, came only after physicists, frustrated by the limitations imposed upon them by the wave length of light, turned to electrons. The wave length of these is very short, so short that this factor so far has not limited the resolving power of the new instrument. Theoretically, the electron microscope is capable of resolving particles separated by only a few Angstrom units. One Angstrom unit is 0.0000001 mm, one ten millionth of a millimetre. Under especially favourable conditions, electron micrographs of useful magnifications of 1½ million diameters have been used to view structure at the molecular level in metallic crystals. Such extreme magnifications are not, at present, particularly informative or useful in the study of cellular structures, but magnification of 100,000 diameters and more are usefully employed in biologic and medical research. The medical and biologic sciences have entered an era in which refined electronic instrumentation and modern scientific techniques and ingenuity have made is possible to gain information about the ultrastructure and microbiochemistry of cells and their organised parts. As information of this type is obtained and effectively correlated, useful knowledge of vitally and medically important cellular processes results.

GENERAL INTRODUCTION.

GENERAL INTRODUCTION.

Microanatomy of the Nephron.

The new technique of electron-microscopy of tissues has made notable advances in our knowledge of the microstructure of the nephron, which in turn has greatly facilitated the understanding of the functional microanatomy and introduced a new and much more accurate concept of renal pathology. This advance began only since about 1950 when it became possible to use the electron microscope for histological study. In 1950 and 1951, the first attempts of microscopists to conquer histology with the electron microscope failed because fixation artefacts were larger than the resolving limits even of the early electron microscopes. Only after Palade (110) introduced buffered osmic acid as a fixative was it possible to eliminate artefacts which could disturb the finest resolvable detail. Also, the technique of thin sectioning used prior to 1953 was inadequate for obtaining conclusive results. Therefore, the early electron microscopic studies of the kidney by Pease and Baker (118) and by Oberling, Gautier and Bernhard (105) although carried out with much ingenuity, led to conclusions which more recent studies, carried out with refined methods, can show to be fallacious.

The first investigators to use these more advanced methods of electron microscopy for studying the kidney structure were B. Vincent Hall of the Zoology Department of the University of Illinois and Fritjof S. Sjöstrand, in association with J. Rhodin, of the Karolinska Institute of Anatomy in Stockholm. To Hall is owed a major part of our present knowledge of the structure of the renal glomerulus, while the Swedish investigators

concentrated their efforts on studying the renal tubules.

The Renal Corpuscle.

The renal corpuscles have been the subject of considerable anatomic and functional interest since their discovery by the great anatomist Malpighi (92). They are a striking example of the unity of structural and functional knowledge for, from the days of their discovery, increasing knowledge of their structure has been paralleled by changing concepts of their function. Malpighi discovered the glomerulus in 1669 by injection of the renal artery, and he considered it to be a gland which gave rise to the urinipherous tube. Schumlansky (141) in 1782, very clearly described the intimate arrangement between glomerulus and tubule and denied the generally held concept that the glomerular tuft emptied directly into the tubule. The classic studies of Bowman (15) in 1842, on the structure of the glomerulus are quite remarkable when viewed in the light of 120 subsequent yeqrs of fairly continuous structural investigation. Bowman injected solutions of potassium dichromate and lead acetate into the renal artery. This gave him the "clearest evidence that the capsule which invests the Malpighian corpuscles[15], in truth, the basement membrane of the urinipherous tube expanded over the tuft of vessels". He noted that all of the blood of the renal artery passed through the tuft and then into a capillary plexus which surrounded the tubule, before leaving the kidney via the renal vein. He described the course of the afferent vessel into the glomerulus where it "perforates the capsule, and, dilating, suddenly breaks up into two, three, four or even eight branches, which diverge in all directions like petals from the stalk of a flower, and usually run in a more or less tortuous manner, subdividing again once or twice as they

advance, over the surface of the ball they are about to form. The vessels resulting from these subdivisions are capillary in size, and consist of a simple, homogeneous, and transparent membrane. They dip into its interior at different points and after further twisting, reunite into a single vessel, which varies in its size, being generally smaller than the terminal twig of the artery. This vessel emerges between two of the primary divisions of the terminal twig of the artery perforating the capsule close to that vessel, and like it, adhering to this membrane as it passes through. It then enters the capillary plexus which surrounds the tortuous urinipherous tubes." He was explicit in his description of the basement membrane which now bears his name. He described the difference in the shape, size, and course of the efferent vessels as they are related to the cortical and juxtamedullary area. He defined and described the two capillary systems of the kidney and proposed that the vessels which carry blood between these two capillary systems be termed a portal system. He speculated upon the function of the Malpighian corpuscle to the effect that it was "an apparatus destined to separate from the blood a watery portion" and "now, however, that it is proved that each one is situated at the remotest extremity of a tube, surrounded by a capsule, formed by its membrane and closed everywhere except at the orifice of the tube, it is evident that conjectures on their use may be framed with greater plausibility."

This ingenious, detailed and very accurate description of the renal corpuscle has not been greatly surpassed till the beginning of the last decade. Investigators in the past century have mainly confirmed Bowman's original description. Some details of the structure of the renal glomerulus, however, have been a matter of great controversy. The points of disagreement among the investigators were summarised by Elias (33) to be concerned with the following questions:-

1. Is the glomerulus covered by epithelium, i.e. by a visceral layer of Bowman's capsule?
2. Are there perforations in the walls of the glomerular capillaries?
3. Is the endothelium of the glomerular capillaries continuous?
4. Are the loops of the glomerular capillaries supported by connective tissue in a manner similar to that of the mesentery which supports the intestinal loops?
5. Do the glomerular capillaries form anastomosing loops?
6. Is the basement membrane of the glomerulus double or single; that is, does it consist of a basement membrane derived from the glomerular epithelium and of another one derived from the glomerular capillaries, or is there only one common basement membrane?

The use of the electron microscope in the past 10 years has advanced significantly our knowledge of the general organisation and structure of the glomerulus beyond that accumulated in the previous 100 years and practically all these problems have been solved. Also notable is that, in general, reconciliation of divergent interpretations regarding the new data has occurred rapidly. It is agreed, too, that variations in structure between all of the mammalian species studied are minimal. Hall's (57,58) original observations were quickly confirmed by other renal electron microscopists (44, 101, 116, 122, 127, 133, 164) and though there is general agreement about the principal features of the microanatomy of the renal corpuscle among them, controversy still persists concerning the arrangement of capillaries in the tuft and on the presence or absence of a third type of cell in addition to the endothelial and visceral epithelial cells, variously called "mesangial", "intercapillary", or most recently, "intraluminal" (76).

It is generally agreed that the renal glomerulus consists of a tuft of capillary vessels inserted into the course of an arteriole; the efferent part of the arteriole is narrower than the afferent. Agreement ends regarding the organisation of the vascular bed between these two limbs. One idea, perhaps the most popular, is that shortly after entering the capsule, the afferent arteriole divides into a number of capillary loops which ultimately gather up to become confluent at the efferent arteriole (31, 158). The other significant concept, is that the afferent arteriole breaks up to form several major branches, each branch giving rise to a lobe or lobule, then subdivides many times forming a freely branching network (68, 165). Recent investigators (16, 33, 35, 58, 59) with their injection techniques and meticulous reconstruction preparations represent the most satisfactory approaches to this problem and their works are substantially in agreement with Johnston's observations (68) of 60 years ago that the capillaries in each lobe form an anastomosing network and that interlobular connections do exist. The minor dispute between the latter workers concerns the degree of anastomosis between vessels, and whether or not true lobulation exists.

Everyone recognises two cell types in the normal glomerular tuft: the epithelium of the glomerulus and a number of cells which are called endothelium. Buday, in 1906 (19) thought that he could differentiate them by their relation to the basement membrane. He believed that the glomerular epithelium lay to the capsular side of the membrane and that all other cells were endothelium. There are, however, some who believe that a third cell type exists in the area of the endothelial cells and have attributed to this "third" cell many of the manifestations of disease as it occurs in the glomerulus.

The question of whether or not a third cell exists in the glomerular

stalk has not been answered by the electron microscopists, but considerable information has been gained regarding the size, shape and position of the endothelial cells. All, however, rather well agree that the endothelial cell is shaped like a tube of a fairly short length. On cross section it is "signet-ring"shaped with a thin layer of cytoplasm extending around the capillary lumen from the mass of cytoplasm which contains the nucleus. The cytoplasm and nuclei of the cell body proper are usually located in the axial region of the capillary loops. The "axis", as well described by McManus (90) refers to those portions of the capillary loops which are located deep within the glomerular tuft in the vicinity of the afferent and efferent arterioles; "peripheral", refers to those portions which have access to the surface of the tuft and face the glomerular capsule. Mueller (101) postulated that the axial stalk consisted of a syncytium of endothelial cells, the peripheral portions of which were hollowed out into spaces forming the capillary lumena. Elias (34) considered syncytial structure a possibility and his diagrams demonstrated this concept very clearly. Other workers (44, 164), however, have described cell boundaries for the endothelial cells.

Elias (34) also analysed the architecture of the renal glomerulus by the statistico-geometrical method and verified his results by three-dimensional models from serial sections. He found that the folds of the basememt membrane enclose a continuous flat and branched mass of cells, tunnelled by hollow cylindrical channels through which blood flows. This tissue, being continuous with the endothelium of afferent and efferent arterioles, is a part of the endothelial system. However, even though blood touches every cell of this tissue, it is not endothelium in the ordinary sense and so he proposed to introduce the term "endenchyma" for this tissue (34).

An endenchyma, he defined, is a continuous mass of cells within a basement membrane; this mass of cells is tunnelled by channels through which blood flows but without basement membranes intervening between the neighbouring channels. The cell bodies, or portions containing the nucleus, lie in the central portions of the stalk, while the outer portions of this cell mass are thin and attenuated and form the attenuated endothelium adjacent to the basement membrane. In reality, his endenchyma is the same glomerular stalk made of endothelial cells that was described by Hall and by Mueller. The importance of his contribution lies in his having focussed attention upon the cell mass - whether or not it is a syncytium - and the relative de-emphasis of the importance of the capillary lumen and its outer wall. He called the entire sheet, which includes endenchyma, blood channels and basement membrane, the "lamina vasculosa glomeruli" (35). He suggested that the position of blood channels in such cellular tissue - on morphological observations - might not be permanently fixed (33) but that these channels change their locations so that under every point of the glomerular surface, blood flows frequently, though not continuously. He subsequently verified his hypothetical assumption by transillumination studies of frog kidneys (35). He maintained that this behaviour of blood channels was possible because of two morphological conditions, namely, because there are no basement membranes or connective tissue elements which might intervene between adjacent blood channels, and because endenchymal cells form a continuous mass in the functional parts of the glomerulus.

The endothelial cell is a relatively large flat cell. Immediately around the nucleus there is a moderate amount of cytoplasm containing the usual cytoplasmic components, including mitochondria, elements of the endoplasmic reticulum with associated ribonucleoprotein (RNP) particles

small piles of Golgi cisternae and vesicles, some free RNP particles, and two centrioles (44). The thinned out portion of the endothelium lying adjacent to the basement membrane consists of two plasma membranes of 150 Å width, separated by a 200-300 Å cytoplasmic space. (100). This membrane in cross section views appears to be discontinuous, as though it were perforated at fairly regular intervals. In tangential views in which the endothelial membrane appears en face, it frequently gives the appearance of "chicken-wire fencing". This appearance initially led Hall to call it the "lamina fenestrata" and later, when there was some question as to whether or not the holes were artefacts or were truly present, he called it "lamina attenuata". Mueller (101) has called it the "endothelial lining network." Rinehart (133) believes that these interruptions represent small segments of cytoplasm devoid of fluid, or cytoplasmic vesicles involved in the transport of fluid, thus giving voice to a possibility that filtration might occur via the endothelial cytoplasm rather than through openings in the endothelium. The size of these openings varies from 500-1000 Å in diameter. The fenestrae are estimated collectively to account for about 30% of the total surface of the endothelial sheets (44).

Farquhar et al (44) have described that in the peripheral parts of the loops where two endothelial cells come together they typically overlap to some degree. Along their line of contact they observed areas of increased density of the immediate adjacent cytoplasmic matrix and pointed to the analogy between this appearance and that of the so-called "adhesion plates" which are present along surfaces of contact of epithelial cells and the "attachment belts" described in vascular endothelia. They have also observed (44) that in the axial or deeper parts of the loops, the endothelial cells were frequently grouped and showed some piling or stratification. The superficial cells could be clearly seen to line the capillary lumen, but the

exact relationship of the deeper cells to the lumen was not always evident. Sometimes, they noticed the deeper cells to show pseudopodia which pushed through the cytoplasm of the superficial cells to establish contact with the lumen. They described "spurs" or processes of varied form arising from the basement membrane in the axial regions which penetrate between endothelial cells. In addition, spongy-appearing areas were often seen between the basement membrane and the deeper cells or penetrating between cells of the endothelium. The spongy areas resembled the basement membrane in density but appeared less compact and more distinctly fibrillar.

In electron micrographs of the normal glomerulus, only Yamada (164), Pak Poy (109), Policard et al (122) and Kurtz and McManus (76) claim to be able to distinguish accurately three cell types - the epithelium, the endothelium and the "third" cell. Zimmerman (165) in 1933, propounced and explicitly described the "third" cell concept of the glomerulus. He rejected the idea that all cells within the confines of the basement membrane were endothelial cells because he found staining differences in this group of cells when he utilized the Golgi-Kopsch stain. In addition to an endothelial cell which bordered the capillary lumen and whose cell body usually lay on the stalk side of the lumen, he found a large pale cell which he thought was a fibrocyte, despite the fact that neither he nor any of the other anatomists had found collagen in the normal glomerulus. Zimmerman believed that 25-35% of the endothelial cells were in reality fibrocytes and that they formed a supporting stalk which stemmed from the hilus of the glomerulus. He labelled this stalk the "mesangium" and the fibrocytes "mesangial cells". He stated that in man, as in all mammals investigated, a little tree of mesangium starts from the hilus, its branches correspond to the lobules and is in contact with all of the capillaries. It was his belief that the mesangium,

together with the basement membrane, formed an important intralobular supporting apparatus, and that without it the capillaries would have nothing to which to hang. Von Möllendorff (95) stated in 1927, that there were no cells in the glomerulus other than epithelial and endothelial. Later, under the influence of Zimmerman's observations, he became convinced that connective tissue did exist. He described (96) a fine connective tissue structure in each glomerulus which branched from the glomerular hilus and to which the capillaries were attached. He, however, expressed doubt that the quantity of "third" cells which Zimmerman saw was really present in the human glomerulus. He accepted Zimmerman's term "mesangium" for this small core of connective tissue and stated that, when ever it lay adjacent to the basement membrane, the mesangium and the membrane could not be distinguished from one another.

The anatomists who studied the glomerulus by the use of silver stains became interested in fibrils extending into the glomerulus from the hilum, apparently continuous with the silver-positive network surrounding the arterioles. Bensley (**9**) thought that these represented a stalk. McGregor (**85**) in 1929, described the continuity of the endothelial cells of the tuft with those of the afferent and efferent vessels. In a second article (**86**), she depicted intercapillary hyaline fibres at the site of the mesangium as one of the changes which occurred in glomerulonephritis, but paid little attention to the stalk or the mesangium concept as such. MacCallum (**82**) in 1934, reported that in the normal glomerulus special attention was required to recognise the few connective tissue cells between the capillaries. He felt that glomerular injury was followed by connective tissue proliferation and, since it was proliferation of the connective tissue rather than an increase in endothelium which eventually caused capillary obstruction, nephritis must be intercapillary rather than intracapillary.

Kimmelstiel and Wilson (72) in 1936, brought the whole problem of intracapillary versus intercapillary disease to the fore by describing hyaline eosinophilic nodules in diabetic kidneys as "intercapillary glomerulosclerosis". Their description rests upon MacCullum's concept of intercapillary disease, and upon Zimmerman's concept that a stalk of fibrocytes exists in each lobule and that there are two basement membranes. Kimmelstiel and Wilson believed that hyaline material extended into the tuft from the efferent vessel and that a broadening and splitting of the capillary basement membrane occurred in addition to an accumulation of intercapillary hyaline fibres. Since the work of Kimmelstiel and Wilson attracted attention to the "intercapillary space", much has been written regarding the diseases which involve it. McManus (89) has consistently and clearly described this space as a position within the confines of the basement membrane, occupied by cells of unknown nature, probably endothelial. He (88) developed and pupularised the Periodic Acid Schiff Stain to demonstrate the glomerular basement membrane and described the capillary loop as curving around a central axis, with the basement membrane covering both the capillary loop and the axial space intervening between the loops. This space always contained either cytoplasm or the nucleus of an endothelial cell. In some cases, the basement membrane appeared thickened at the site in which it lay adjacent to the axial space, producing one variety of axial thickening. McManus stated that the nuclei of these axial cells stained like endothelial cells, but he could not identify them with certainty and called them "intercapillary" or "third" cells, feeling that they were probably the cells described by Zimmerman as fibrocytes or by Goormaghtigh (53) as muscle cells which controlled glomerular tone.

Most electron microscopists could not detect this "third" cell. Hamada (164)

was the first of them to describe its existence; he called it intercapillary cell. He also commented on the spongy fingers of basement membrane material which are present at the sites where the basement membrane is in contact with the intercapillary or "third cell". Thickening of the basement membrane at this point has been noted by all of the micro-anatomists. Finger-like projections extending from the basement membrane around and through the cells of the stalk are present in the electron micrographs of most observers, although Yamada was the first to bring attention to them. Yamada mentioned that the basement membrane was often lacking or incomplete at the inner (stalk) side of the capillary loop, and, the epithelium might then come into direct contact with the endothelium or "third" cell. Such discontinuity in the basement membrane has been subsequently reported only by Policard et al (122) In the cytoplasm of the intercapillary cell, Yamada (164) recognised mitochondria, reticulum, vesicles, and numerous delicate filaments which suggested a similarity to smooth muscle cells. The characteristic fibrillar nature of these cells permitted Yamada to distinguish them from endothelial cells. He described small processes which extended from these cells to the capillary lumina, there forming "intercapillary colliculi". Electron micrographs of other workers do not show distinctions between endothelial cells and intercapillary cells which Yamada describes, and a review of his published electron micrographs by Mueller (100) showed the differences between endothelial and "third" cells to be less apparent than his description indicated. However, Kurtz and McManus (76), studying the human glomerulus by the electron microscope repeatedly observed the continuity of basement membrane enclosing many capillary lumina and isolated masses of endothelial cells. At the extreme periphery of the glomerulus only the basement membrane was noted to encircle the capillary lumen in 360° fashion, thus confirming that

the endothelial cells are axially disposed. They described that the cytoplasm of endothelial cells related to the lumen was usually less dense than its neighbour between lumina and explained the denseness of the axial cells to a lesser degree of hydration than their luminal counterpart.

Considerations raised by a knowledge of the embryology of the glomerulus (1, 25, 67) and by staining properties of the diseased glomerulus (8, 10, 69 70, 71, 90) led the light microscopists to a concept that the apparently single basement membrane of the normal glomerulus was in reality composed of two basement membranes - one continuous with the basement membrane of Bowman's capsule (the subepithelial membrane) and the other continuous with the basement membrane of the arterioles (the capillary membrane). These were thought to be indistinguishable in the normal glomerulus because of their proximity to each other, but were felt to be identifiable when separated by disease (69, 71). Zimmerman (165) stated that he was able to distinguish two basement membranes, which together with a thin optically-inseparable endothelium form the capillary wall. Allen (1) has based his concepts of glomerular disease upon the postulate that two basement membranes exist.

Modern techniques of electron microscopy have established that there is actually but ONE basement membrane in the normal glomerulus, and ALL electron microscopic studies of the glomerulus have agreed upon this point. Hall (57) described it as a thin sheet of dense homogeneous material between the endothelium on one side and the epithelium on the other, and called it the "lamina densa". He believed in his earliest micrographs (57) that he could identify tiny pores in it, but with refinement of his techniques, however, pores have not been discernible, and other electron microscopic studies have confirmed that it is the only continuous structure of the wall of the glomerular tuft (17, 116, 127). Its thickness from the outer endothelial cell

membrane to the epithelial cell membrane limiting the foot processes has been variously reported by different authors (Table 1).

Table 1.

Author	Thickness of Glomerular Basement Membrane	Species
Hall (57)	600 - 800 Å	Rat
Rinehart (133)	800 Å	Rat
Rhodin (127)	1250 Å	Rat
Dalton (28)	1600 Å	Mouse
Farquhar et al (44)	1000 - 1500 Å	Rat
Yamada (164)	800 Å	Mouse
Mueller et al (101)	2000 - 2400 Å	Man and dog.

However, it is agreed that it is slightly thicker in the human glomerulus than in glomeruli of most laboratory animals, and that it is thin in infants and gets slightly thicker with age (40).

Rhodin (127) described the basement membrane as having three layers - a central dense, and two outer less dense, layers. The less dense layer of basement membrane adjacent to the epithelial processes lead Pease (115) to postulate that the foot processes of the epithelial cells were actually embedded in a cement substance of the basement membrane. Yamada (164) felt that faint membranes extended from the foot processes to the inner layer of the basement membrane. Such connections as these between epithelial foot

processes and the basement membrane have not been described by other microscopists. Policard et al (122) thought that an intermediate space existed between the basement membrane and the foot processes, but they were actually speaking about the light external layer of the basement membrane. Farquhar et al (44) noticed that the width of these lighter areas varies noticeably with the amount of extraction incurred during fixation and embedding; and maintained that in optimal preparations the lighter areas are extremely thin, the dense layer nearly filling the space between the endothelium and epithelium.

The glomerular basement membrane consists of a continuous layer of moderately dense material, which appears homogeneous in unstained preparations, with no apparent fibrils (6,33,34,45, 57,58 ,101, 105,122 , 133). Some investigators have suggested the existence of a fundamental feltwork within the basement membrane whose density was not so adequate for convincing photography (11, 94 , 127 , 164). However, after staining with heavy metals (lead hydroxide, uranyl acetate or phosphotungstic acid) it shows a faintly fibrillar structure produced by the presence of fine fibrils (30-40 Å in diameter) which appear to be embedded in an amorphous matrix (44 , 75, 76 , 144 149). In addition, Farquhar et al (44) have described bundles of distinct fibrils (110 Å in diameter) in the narrow space between the basement membrane and the endothelium. They believed that these are produced by the endothelium and might ultimately be incorporated into the basement membrane. Both these fibrillar components are quite distinct from mature collagen or reticulin fibrils which are well known from histochemical (135) and electron microscopic evidence to be absent from the normal mammalian glomerulus.

The basement membrane of Bowman's capsule is believed to be continuous

with the basement membrane of the proximal convoluted tubule and related in some fashion, which is still controversial, to the arterioles and capillaries at the hilum of the glomerulus. It was shown by light microscopists to have two layers - an outer reticular zone and an inner clear homogeneous zone (25,26,49,82,85,91). The reticulum fibrils embedded in the external surface appear to be part of the overall supporting structure of the kidney, and not an inherent part of either this membrane or its continuation as the basement membrane of the tubule (25,91). In this thesis, the term "capsular basement membrane" will be used to describe the homogeneous part of that membrane only.

Bowman (15) and Hall (58) stated that Bowman's capsule is pierced by the capillary tuft, and "was certainly not reflected over the vessels (15)". Hall (57,58) maintained that the capillary basement membrane is continuous with that which surrounds the hilar vessels and is not continuous with that of the capsule and the tubules. However, neither of these authors offered tangible evidence to this effect. McGregor (85), Bohle (12) and Elias (34) felt that a basement membrane from the afferent and efferent vessels joined with the basement membrane of Bowman's capsule to form jointly the glomerular basement membrane. MacCullum (82) and Mueller (100) described the capsular basement membrane as turning back upon itself at the hilum to become the glomerular basement membrane.

The general appearance of the substance of the capsular basement membrane is similar to that of the glomerular basement membrane, except that it is coarsely laminated (76,100). By the electron microscope, it has been shown to be a laminated, dense material of a fibrillar nature with a multitude of fuzzy fibrils on the external surface, in keeping with the description of older anatomists.

Bowman's capsule is lined by a layer of epithelial cells which are continuous with those lining the proximal convoluted tubule and those covering the glomerular capillaries, but differ markedly in appearance from either. They are usually flattened cells, with distinct cell borders, that bulge out in the portion that contains the nucleus. Electron microscopic examination of these cells shows them to have a few mitochondria scattered throughout the cytoplasm but no intricate organisation of internal structure such as characterises the cells of the proximal tubule (101, 164).

The glomerular capillary basement membrane is covered towards the urinary space by epithelial cells continuous with those lining the capsular basement membrane. These glomerular epithelial cells were first described by Gerlach in 1845 (47); Bowman (15) thought that the glomerular capillaries were naked and "uncovered by any structure". Since then, however, they have been studied by most glomerular microscopists and now are recognised to be a very bizarre trabeculated cell whose shape defied visualisation and accurate description. Nassbaum (103) in 1866 noticed that they project steeply into the capsular space and observed their deeply indented nuclei. Clara (22) called them "epicytes", a name subsequently used by Kulenkampff (74). Von Möllendorff and Bargmann (5) observed long, slender processes of these cells when preparations were stained with iron haematoxylin. Bargmann also observed that a single cell may extend across a crevice between two capillaries. Zimmerman (165), using the light microscope, prepared the best drawings of these cells. With silver impregnations he demonstrated their long penniform processes. He described them as similar to twigs of fir trees, arborising over the free surface of the capillaries in the tuft. He called them "deckzellen" or surface cells. Kulenkampff (74) in 1954, utilised Zimmerman's Golgi-Kopsch stain to study some of the reactions of

these cells. He obtained even better staining of their processes by using molybdic haematoxylin.

The unusual nature of these cells has been revealed by electron microscopy (57, 101, 116, 164). They are a fairly large cell with abundant cytoplasm. The nucleus is typically found near the cell surface facing the urinary space and has deep incisions. In the normal glomerulus the cytoplasm extending away from the nucleus gives rise to many arms which often exhibit a highly ruffled contour. Each arm, in turn, is elaborately organised into a number of secondary branches, small finger-like processes, that interdigitate on the capsular surface of the glomerular basement membrane with similar processes from other arms of an adjacent or of the same cell. Hall (58) has named this cell "podocyte", its broad extensions trabeculae and the finger-like terminations "pedicels"or foot processes. This highly organised layer of epithelial cells has been found to cover the glomerular capillary walls of human, other mammalian, avian, amphibian and marine elasmobranch kidneys, In mammalian glomeruli, each individual pedicel may be 1-1.5 μ in length, about 2.200 Å in width and about 6000 Å in height (101). Each foot process typically has a narrow stalk at the point of origin from its trabecula, but expands into a broader base in contact with the basement membrane. As a result of this arrangement, the space between the foot processes forms narrow slits, called by Hall "slit-pores", near the basement membrane and rapidly increases in width towards the urinary spaces. Hall (60) gave an approximate width of the slit pores of 100 Å, most other authors found them slightly wider, 200-300 Å (101). Yamada (164) in 1955, described a thin line with ill-defined limits bridging the narrowest point of the gap between foot processes and called it "filtration slit membrane". This filtration slit membrane appears in Rhodin's (127) and Policard's (122) pictures but is not

mentioned in the text. Pease (116) confirmed its presence and concluded that the line represents the free margin of a "cement substance", whereas Yamada has considered it as the outer layer of the cell membrane. In 1961, Farquhar and her associates (44) described a more complex organisation in the epithelial slits. They observed thickening of the opposed cell membranes of the foot processes, backed by an increased density of the immiately subjacent cytoplasmic matrix. They also detected a linear accumulation of dense material bisecting the "filtration slit membrane". They believed that there was a striking similarity between the structural pattern described in the slits and that encountered in terminal bars and desmosomes. They suggested that these sites are functionally as well as morphologically specialised.

Most microscopists feel that filtrate could pass from basement membrane into the capsular space without traversing any epithelial protoplasmic barrier. In the electron micrographs of most authors there are views in which a trabecula overlies some of the foot processes. Oberling, Gautier and Bernhard (46,105) were so much impressed by this labyrinthine appearance that they described the space as an "appareil lacunaire peri-capillary" or pericapillary sinus and suspected that this may have some function in the regulation of filtration. Policard et al (122) were also impressed by the appearance of a pericapillary sinus and postulated that if the foot processes should change in size, this space or sinus might act as a submicroscopic sponge.

Most observers have commented upon the presence of mitochondria, Golgi bodies, vesicles and other cytoplasmic inclusions in the glomerular epithelial cells. No special study of these intracytoplasmic structures has been reported except in 1961 by Farquhar and her colleagues (44). They reported that the internal organisation of these cells was as complex and intriguing as

their external features. They divided the cell arbitrarily into three zones: the cell body or perikaryon near the nucleus, the intermediate cytoplasmic zones constituting the trabeculae, and the peripheral foot processes. In the perikaryon, near one pole of the nucleus, there is usually a large centrosphere region with numerous piles of parallel, closely packed, smooth surfaced Golgi cisternae surrounded by swarms of small vesicles (600-800 Å) and occasional larger vacuoles. Both smooth surfaced (without attached RNP particles) and rough surfaced (associated with RNP particles) elements of the endoplasmic reticulum are present in abundance and are frequently seen in continuity with Golgi elements. Mitochondria, and multivesicular bodies (first described by Yamada (164)) are also typically found in this region. The intermediate cytoplasmic zones contain a well-developed endoplasmic reticulum composed of vesicular and tubular elements of both the smooth and rough surfaced varieties. There are also variable numbers of free RNP particles. In these intermediate regions one often encounters rather remarkable structures which represent large distended cisternae of the endoplasmic reticulum, measuring up to 1.5 mμ in diameter. They are limited by rough surfaced membranes and are in direct continuity with the more common flattened cisternae. The intermediate cytoplasmic areas also contain numerous small vesicles and occasional multivesicular bodies, together with a few vacuoles and dense bodies. The vacuoles are membrane-limited and have a content of low density: they range in size from 0.2 to 1.0 μ. The dense bodies are also limited by a membrane; they range in size from 0.1 to 0.2 μ.

Farquhar et al (44) described that the membrane of these epithelial cells, though similar to other cell membranes, is much thicker. It is composed of two dense layers (about 40 Å) separated by a space of lesser density (about 30 Å), having a total thickness of about 110 Å . After lead staining, its density

was observed to be conspicuously higher than that of the endothelial cell membranes.

Perhaps one of the most important and fascinating results of recent electron microscopic research on the glomerulus has been the clearing up of the structural basis of glomerular ultrafiltration. It is generally accepted that urine formation begins with the passive process of ultrafiltration. The original concept was formulated by Ludwig in 1844 (81) and elaborated by Cushny (27), but fully satisfying proof of this theory was only given when Wearn, Richards and others after them, adopted and used new quantitative physiologic and microchemical methods for the analysis of glomerular filtrates. The original idea of a protein-free filtrate has been modified in recent years to take into account that small quantities of protein normally leak into the capsular space and are reabsorbed by the tubules. Available evidence (7, 14, 55, 93, 159) suggests that the normal glomerulus is to some degree permeable to molecules with a mean diameter in the range of 50-100 Å, but there is apparently progressive restriction to passage with increasing molecular weight and average diameter. As a result of extensive permeability studies, Pappenheimer and his associates (113, 114) have postulated that the wall of glomerular capillaries consists of a membrane containing pores with an effective diameter of about 60-90 Å. Pappenheimer, however, considered the possibility of a more complex geometry for the filtration barrier and suggested that the channels might be formed by a gel structure or a fibrous structure (112). Whatever the structural nature of the filtration membranes, it would present the same barrier to hydrodynamic flow and diffusion of solutes as would uniform cylindrical pores of radius 30 Å. They have explained the higher filtration of glomerular capillaries by

assuming that the pores represent collectively 1-2% of the total surface of these vessels as opposed to 0.2% in the case of muscle capillaries. Recent electron microscopic studies indicate, however, that the two types of capillaries differ significantly in structure; in the muscle capillaries (98, 111) the endothelium forms a continuous layer without fenestrations and the outer surface of the basement membrane is in contact with the connective tissue elements. Pores of the expected dimensions are not present in the wall of either type. In some early electron microscopic studies, the newly discovered fenestrae in the endothelium were identified as the pores postulated by the filtration hypothesis, but this interpretation was quickly discarded when it became apparent that the fenestrae are considerably larger than the hypothetical pores and interrupt only one of the three layers of the capillary wall.

Both the endothelial and epithelial layers of the glomerular capillary are discontinuous. Attempts to demonstrate regular porosity in the basement membrane have met with general failure (121). This failure to establish the presence of any regular porous structure in the basement membrane, led Hall (60) to formulate his concept of filtration through epithelial "slit-pores". According to this hypothesis, the size-limiting structure is the "slit-pore" formed by the close approximation of adjacent foot processes. These slits were presumed to impose a more precise restriction than does the basement membrane. Hall could not bring out any experimental proof of his hypothesis. Against the slit-pore hypothesis, is the fact that the slits occupy a significantly greater precentage of the total area of the capillary wall than the pores postulated by Pappenheimer et al (about 20% as compared to 2%).

The majority of glomerular electron microscopists (115, 127, 164) have

THE UNIVERSITY *of* EDINBURGH

assumed that the glomerular basement membrane acts as the main filter, because it is the only continuous layer found in the capillary wall; proof for this came only very recently. In 1961, Farquhar, Wissig and Palade (44) reported convincing evidence on the structural basis of formation of the glomerular fluid. They injected the electron dense ferritin molecules, as a marker, intravenously in rats and visualised directly their pathway across the glomerular capillary wall. They found that the concentration of ferritin molecules fell off sharply at the level of the basement membrane, with retention of most of the tracer molecules in the capillary lumen. This finding amounted to a direct demonstration that the filtration barrier is not freely permeable to molecules about 100 Å diameter. A small percentage of ferritin molecules did penetrate the basement membrane and were subsequently picked up by the epithelium, indicating that the basement membrane is not a perfect filter. It was assumed that it apparently allows continuous passage of protein molecules (diameter less than 100 Å) in relatively small amounts, which, at least in part, are subsequently removed from the filtrate by the pinocytic activity of the epithelium. It appeared, therefore, that one of the functions of the epithelium is to "check" the glomerular filtrate, a view strongly supported by the finding that this activity is greatly enhanced when appropriately challenged, i.e. when increased quantities of protein appear in the glomerular filtrate as in the nephrotic syndrome (42). After its incorporation into the epithelium, the ingested protein is apparently segregated into multivesicular bodies and vacuoles, which appear to undergo condensation into dense bodies. These authors (44) have postulated that the desmosome-like structure which they have described in the slits might function as a seal for the larger molecules, thus providing maximal

opportunity for protein which leaks through the basement membrane to come into contact with the epithelial cell surface and be incorporated by pinocytosis. The findings of these authors repudiate the "slit-pore" hypothesis of Hall (60) since no direct evidence for any size-limitation introduced by the slit pores was found; there was no piling of the marker at the slit intraite, although molecules of the ferritin size are expected to be extensively retained by the glomerular filter.

These recent results indicate that the formation of glomerular fluid is a more complicated process than passive filtration through a membrane provided with rigid pores. They suggest a definite role for each of the components of the glomerular capillary wall in the filtration process: the basement membrane as the principal filter; the epithelium as its monitor; and the endothelium as a possible valve, which by varying the number and distribution of its fenestrae controls the area of the filter directly exposed to the blood plasma (44). The principal filter (the basement membrane) does not appear to be a simple sieve, but presumably a gel-like structure with fine fibrillar components, embedded in a matrix still amorphous at the present level of resolution. It thus comes close to Chinard's representation (20, 21) of the structure of the capillary wall and different from Pappenheimer's (113, 114) and Smith's (147) concept of a sieve-like capillary wall provided with pores; all these hypotheses being primarily based on permeability studies and conducted by renal physiologists without taking into account the recent morpholotical information on the glomerular capillary wall. The work of Farquhar and her colleagues (44) has shown that the tracer molecules move through a yielding substrate in the absence of permanent pores or channels.

The Renal Tubules.

The electron microscopic study of the renal tubules was practically only reported from the Karolinska Institute, Stockholm. In 1953, Sjöstrand and Rhodin (146) published the first electron microscopic description of the proximal convoluted tubules. This part of the renal tubule was the site of further studies by Rhodin and formed the basis of his thesis in 1954 (126) and a paper given by him at the Stockholm Conference on electron microscopy in 1956 (128). In 1958 he reported a detailed electron microscopic study of all types of the renal tubules (129), and reviewed his works on electron microscopy of the kidney (130). The following description of the renal tubules is based largely upon Rhodin's detailed studies.

The lumen of the proximal convoluted tubule is usually closed, and the central part of the tubule is thus occupied by a multitude of densely-packed microvilli. Each microvillus has a length of about 1 μ and a width of about 700 Å. There are roughly 215 microvilli per square micron in the first part of the proximal tubule. However, the number of microvilli decreases towards the thin segment of Henle's loop. A distinct cell membrane with a thickness of 40 Å separates the lumen of the tubules from the interior of the microvillus which is connected freely with the rest of the cell cytoplasm. The interior of the microvillus is structureless, and less dense than the rest of the cell cytoplasm. At the bases of the microvilli, small holes open into coiled tubular invaginations of the surface membrane of the cell. The width of each invagination is about the same as that of a microvillus, whereas the length is shorter. The invaginations are sometimes widened to small bulbs, but they do not seem

to be connected with the large vacuoles which occur in the apical cytoplasm. The nearby large vacuoles correspond to autofluorescent granules which Sjöstrand (145) observed in sectioned and unstained material, prepared by the freeze-drying technique. In close relation to the fluorescent vacuoles, are encountered large electron-dense granules, which often display a system of concentrically arranged thin membranes.

In the cells of the proximal tubule, there is also a Golgi zone, lying like a halo around the apical part of the nucleus. It is composed of a system of paired membranes parallel to each other, with small vesicles scattered either between the paired membranes or peripherally. Rhodin suggested that one task of the Golgi zone is to provide the necessary supply of mitochondrial precursors.

The mitochondria of the cells of the proximal tubule occupy the largest part of these cells and form elongated, sausage-like, coiled bodies. The fine structure of a mitochondrion consists of a surrounding triple-layered membrane, and a system of triple-layered membranes arranged parallel to each other in the interior of the mitochondrial body, maihly perpendicularly to the long axis of the mitochondria. Rhodin suggested that these inner membranes or plates presumably represented the site of the enzyme molecules or the surfaces upon which part of the enzymatic activity can occur. He maintained that this fine structure was probably necessary for the normal function of a mitochondrion.

The basal part of the tubule cell is provided with a multitude of cytoplasmic lamellae. Rhodin described them as an arrangement resembling the cone-shaped cogwheel in a differential gear of a car; He considered that the different tubule cells fit together by interlocking of their basal lamellae. This organisation of the bases of the tubule cells, means that

the basal surfaces of the cells are tremendously increased, as a result of this lamellation, the basal cell membrane will be encountered also at varying distances within the cell body, as seen in a section of a cell. The mitochondria are almost exclusively located within the basal cytoplasmic lamellae and this was considered by Rhodin as a strong support to his idea that the multifolded cell membrane is designed in this way in order to increase the surface upon which enzymatic activity can occur. He drew attention to the similarity between the lamellation of the tubule cells and the trabeculation of the glomerular epithelial cells, but he could not define that these lamellae are divided into smaller subunits like pedicels. Rhodin noted that the interlocking of the cells was not well developed at birth, but continued for 2 weeks after birth when it seems to reach its full development at the same time as the nephron functions. In glomerular fish (Lephius piscatorius) the proximal tubular cells lack these inflections of the basal cell membrane, whereas glomerular fish (squalus acanthias) do possess these structures.

The sides of the upper portion of the cells are smooth, without the typical basally located lamellae. In transverse sections through this region each cell displays a multi-angular shape, while in sections through the basal part, it appears as an irregular, multipointed star. Close to the surface of the cell, each cell is strengthened and attached to another by the so-called"terminal bars" which represent a local thickening of the cell membrane, oriented as a coopering band around the top part of the cell, and almost completely filling the space between the cell borders, a space which averages 70 Å and probably is composed of at least two layers of lipoid molecules.

The nucleus is located in the centre of the basal part of the cell.

It does not go into the cytoplasmic lamellae. A triple-layered membrane with a thickness of about 250 Å surrounds the nucleus, One or several nucleoli are present, composed of fine granules, as is the rest of the nuclear content.

The structure of the cytoplasmic ground substance is finely granulated, with particles of RNP averaging 160 Å .

The cells of the descending limb of Henle's loop are different from those of the proximal part of the convolution. The number of microvilli decreases, the mitochondria are less abundant and shorter, more like speres; the cytoplasm itself is less dense and the basal cytoplasmic lamellae are rare, sometimes absent (128).

As in most squamous epithelia, the nuclei cause the top part of the cell to bulge in the tubular lumen. The surface of the cell is fitted with tiny microvilli, spaced apart at a distance of about 0.1 µ. The surface of the villi and that of the areas between them is covered with a single membrane, 70 Å thick, Invaginations of the membrane do not seem to occur in this part of the nephron. The cytoplasm is pale and holds only a few scattered mitochondria together with a very restricted Golgi zone, features encountered mostly in the naighbourhood of the nucleus.

The cytoplasm of the squamous cells is extremely attenuated, the height of the thinnest parts averages 0.5 µ. The attenuated cytoplasm is composed of lamellae arranged in such a way that the cells of the thin segment resemble starfish with a multitude of arms resting on the basement membrane. The arms do not have a straight course but a highly curved and

wavy one, and the arms or lamellae of one cell interdigitate with lamella of neighbouring cells, in a manner similar to the arrangement of the cells of the proximal tubule, only with the exception that the cells of the thin segment are flattened down so that the upper part of the thick portions of the cells are not in contact with each other. The cytoplasmic lamellae of the cells of the thin segment are rather narrow and frequently contain mitochondria. The lamellae are surrounded by a cell membrane which is provided with terminal bars near the lumenal surface. The organisation of the attenuated processes of the cell body, interdigitating and resting on a basement membrane is almost identical with the picture of the pedicels of the glomerular epithelial cells.

Following the classification suggested by Möllendorff in 1930 (96), the distal tubule includes

1. The ascending thick limb of the loop of Henle.

2. The macula densa, in contact with the afferent arteriole.

3. The intercolated portion: the convoluted part in the cortex between the macula densa and the collecting tubule.

In light microscopy, the distal tubule is usually easily differentiated from the proximal tubule owing to

a) a clear free lumen.

b) absence of a brush border.

c) very dark striations in the basal two-thirds of the cells.

d) an abundance of nuclei, compared with the proximal convolution, located in the apical cell zone close to the lumen.

By the electron microscope, in the first part of the ascending limb of Hanle's loop the cells are cuboidal and characterised by a rather

clear cytoplasm resembling that of the cells of the thin segment. However, as the neighbourhood of the glomerulus is approached, the cells become more columnar, the cytoplasm darkens and the mitochondria increase in number and length. The long mitochondria are particularly typical of this part of the nephron; they contribute to the marked basal striations, observed even in the light microscope. The diameter of the tubule is larger than in the proximal convolution and a wide free lumen is always present.

There is a complete lack of a true brush border, but a few small microvilli with a length of about 0.4 μ are scattered over the surface of the cells of the distal convoluted tubule. The microvilli, as well as the free cell surface, are covered by a cell membrane 70 Å thick.

The luminal third of the cell is characterised by an abundance of small vesicles. Those located close to the surface of the cell are now and then in open connection with the tubule lumen. A certain number of fluorescent granule vacuoles as well as large electron dense granules are present, but to a lesser extent than in the cells of the proximal tubules.

There is a Golgi zone with a location and fine structure similar to that which has been described in the proximal convolution. However, the vesicles seem to be larger in the distal convolution, almost achieving the size of the fluorescent vacuoles. A certain number of microbodies occur but with less abundance than in the proximal convolution.

The mitochondria of the cells of the distal convoluted tubule have a length of about 3 μ or more and their width averages 0.3 μ in one direction

whereas their thickness amounts to or even exceeds 1 µ. In consequence of this the mitochondria have a closer resemblance to flat icecream bars than to sausages. This is true for the mitochondria which occupy the basal two-thirds of the cell. In the luminal third, small spherical or slightly oval mitochondria are seen scattered between the abundant small vesicles already mentioned. The basal mitochondria are tightly packed, sometimes so markedly packed that the interposed narrow strands of cytoplasm are hard to distinguish. The fine structure is almost identical with that described for the mitochondria of the proximal convolution except that the inner, triple-layered membranes or plates are sometimes seen oriented at various angles to the long axis of the mitochondrian, which seems to indicate that their three-dimentional orientation is a helix rather than simple straight plates across the interior of the mitochondrial body.

The basal part of the cell displays cytoplasmic lamellae similar to those found in the proximal convolution, although the lamellae seem to be narrower, deeper or of greater abundance in the distal convolution. In addition, terminal bars are present, with a structure and location identical with that already described for this cell component in the proximal convolution. The nucleus is placed in the luminal part of the cell and bulges the cell surface into the lumen. The cytoplasmic ground substance is built up of RNP-granuoles, which are abundant in the luminal part of the cell, but almost absent in the basal part, within the cytoplasmic lamellae.

The collecting tubule is wide, and as the lining cells are rather shallow, this opens a wide and free lumen. The cells are mainly of two

types, the light cells and the dark or intercolated cells.

The light cells have a loose cytoplasm due to a scarcity of cell organelles such as mitochondria and RNP granuoles. The nucleus has a prominent location in the cell, leaving but little cytoplasm free. Between the cells are deep indentations of the surface, which sometimes, almost reach the basement membrane. A membrane with a thickness of 70 Å covers the surface of the cell as well as the scattered, few and short microvilli which emerge at a distance of about 0.5 µ from each other. Some small vesicles occur in the apical part of the cell, occasional fluorescent granuole vacuoles and large electron dense granuoles may be encountered. The mitochondria are few and located mostly in the basal part of the cell. Their width averages 0.3 µ . The fine structure is identical with that of mitochondria in the proximal tubule. Microbodies have not been encountered in the light cells. The Golgi zone is small but of regular structural appearance. It generally surrounds the upper part of the nucleus, but it may also be more apical in position. The basal part of the light cell shows a few shallow inflections of the cell membrane, which may be interpreted as cytoplasmic lamellae or basal ridges. However, they seldom interdigitate with similar structures from neighbouring light cells, probably because they are too short and shallow to penetrate underneath the cells and become intercolated. Lapp (77) in 1960 noted that the form of the free surface of these cells and the extent of the lumen of the collecting tubules depended upon the functional condition of dehydration or diuresis.

The dark cells of the collecting tubules were first well described by Andrejewitsch in 1919 (2). This author also noticed a difference

in the shape of these cells, and concluded that this was due to different functional states in a secretory process. In 1957, Oliver and his co-workers (108) by micro-dissection and light microscopic studies, recorded an increased number of these dark cells and a decreased number of the light cells in the collecting tubules as a result of potassium depletion in rats; a structural change associated with an inability to concentrate the urine. In 1957, Clark (23) in an electron microscopic study concluded that the light and the dark cells of the collecting tubules are actually identical cells but with a variation in density because of the difference in functional states. In 1958, Rhodin (129, 130) and in 1960 Lapp (77) described the electron microscopic appearance of the dark cells in detail. They noted that they have a very dense cytoplasm mainly due to an abundance of RNP granules, each with a thickness of 160 Å. The mitochondria are more numerous, larger and with a thicker matrix than the light cells. A greater richness of compact cytosomes of varying sizes contributes to the dark appearance (, 77). The basal cell membrane is more deeply folded in. Rhodin (129, 130) noticed that these cells show a certain variation in height and form, sometimes being taller than the light cells and sometimes more shallow with a tendency to extend underneath the light cells, between their cell body and the basement membrane and for this reason they have sometimes been called the "intercolated cells." The dark cells do interdigitate with the light cells, but only in the periphery, where the cytoplasmic lamellae are long enough to interlock with each other. This is especially so when part of the dark cell is intercolated between the base of a light cell and the basement membrane. The luminal surface has an abundance of short microvilli, all surrounded by a cell membrane. Lapp (77) noticed a definite change in the

appearance of these cells in different functional conditions; he described that both in the lightly and strongly concentrating states of the kidney, these dark cells bulge for a great distance in the lumen and the compact cytosomes appear in greater numbers.

The main difference between Rhodin's and Lapp's description of the dark cells of the collecting tubules is their location along the collecting tubule. Rhodin mentioned that close to the point where the distal tubule connects with the arched collecting tubule, the light cells are rare and the dark cells abundant; then the dark cells become more and more rare as we move distally along the collecting tubule, and in the renal papilla, the light cells predominate. Rhodin considered that the dark cells are similar in appearance to the cells of the distal convoluted tubule and hypothesised that they represented a certain variety of cells of the distal convoluted tubule which are distributed along the collecting tubule towards the papilla at a decreasing frequency. For this reason he assumed that they share with the cells of the distal tubules the function of secretion of ammonia and the acidification of urine. Lapp, however, always failed to find the dark cells in the collecting tubules in the outer medulla and in the inner half of the inner medulla; he always found them to be well localised to the segments of the collecting tubules in the outer half of the inner medulla. He is also convinced that the morphological similarity of the dark cells to the cells of the distal convoluted tubules is slight. However, he still ascribed to them the possible function of ion exchange and regulation of the acid-base equilibrium.

Within the past few years reports have started to appear indicating an attack upon glomerular pathology by the electron microscopists. Farquhar (40 , 43) and Spiro (149) in the States and MacDonald (83,84) in Scotland have begun to describe the anatomic differences between the nephroses, in which epithelial cell and basement membrane alterations are present, and the nephritides in which endothelial and basement membrane changes can be seen. Piel et al (120), Ried (131) and Spargo et al (148) have made attempts to study experimental renal disease with the electron microscope and much work in this field is undoubtedly forthcoming. Newer staining techniques have recently been employed and in the years immediately ahead much information and more accurate redescriptions of the human renal pathology should be available through the use of percutaneous renal biopsy and electron microscopy.

In this thesis an attempt has been made to restudy the micro-anatomy of the nephron and to clarify particularly the few points on which electron microscopists still do not agree. To achieve this, a study of the development of the renal glomerulus was found very valuable in addition to the study of the mature mammalian glomerulus.

Though some work has been made to elucidate the process of glomerular filtration through the use of electron microscopic technique no work has yet been reported on the value of this method of study in clarifying the function of the tubules. The recently described hair-pin countercurrent theory for concentrating the urine and the mode of action of the antidiuretic hormone (163) has been considered to be the most fascinating new development in the field of renal phsyiology in the last decade. This theory, however, still has differing versions in certain

details and renal physiologists are trying to construct experiments to solve the few mysterious points in this theory. It was thought that an electron microscopic study, by revealing the ultrastructure of the different cells of the nephron at the molecular level, might be of value in this respect when applied to the kidney in different functional states of forced hydration and forced dehydration.

The study of renal physiology through a change in morphology during the performance of specific functions has always been a fascinating approach, and electron microscopy has made it possible. The antidiuretic hormone and the parathyroid hormone, two hormones known to excite their main physiological functions in the body through their effect on the renal tubular handling of water in the first instance and of phosphates in the second, were chosen for such a pioneer study, which if successful would add a new useful technique to the various methods physiologists practise in their attempts to clarify the multiple, complicated, very important and vital functions of the kidney.

Similarly, a study of the effects of electrolyte imbalance on the renal fine structure and function seemed fascinating. Depletion of potassium and magnesium, the two main intracellular cations, were investigated. In the clinical and experimental potassium depletion, the kidney is known to show morphological as well as functional changes. Since themain functional abnormality in this condition is loss of the power of the kidney to concentrate the urine, it was thought that a study of the renal ultrastructure might add weight to whatever findings and conclusions might be arrived at by the electron microscopic study of the mechanism by which the kidney is able to concentrate the urine. Magnesium is known

to behave like potassium in certain aspects and like calcium in other aspects of body metabolism. The electron microscopic appearance of the kidney in experimental magnesium depletion and its comparison with the appearances in potassium depletion on the one hand and with those resulting from administration of the parathyroid hormone on the other hand, was therefore contemplated to be of value.

One of the main subjects in which I am interested is diabetic nephropathy. Its study constituted my M.D. thesis and appeared in a number of publications (36, 37, 38, 138, 139). It was natural to apply electron microscopic technique, once I learned it, to carry further my previous studies on the diabetic renal lesions, particularly in relation to their pathogenesis. This was done partly by studying glomeruli from young human diabetic patients with normal renal histology on light microscopy and by the electron microscopic study of the renal lesions which result from prednisolone administration to adult rabbits, and subsequently comparing the results obtained with the known theories of the pathogenesis of diabetic nephropathy.

METHODS.

METHODS.

Tissue Preparation for electron microscopy involves fixing (hardening and preserving) the tissue, dehydrating it and infiltrating it with a substance that can be hardened or set to give a material suitable for cutting thin sections.

Human material was obtained by percutaneous needle biopsy, using a Franklin-Silverman biopsy needle, and animal material was obtained by exposing the kidney immediately after killing the animal with a blow on the head, thus avoiding exanguination or the use of an anaesthetic which might influence the appearance of the tissue at a final structure level.

To avoid cytolytic and post mortem changes, the tissue was brought into contact with the fixative in less than 30 seconds after removal from the body by transferring it into one or two drops of the fixative placed in a Petri dish. The tissue was then cut quickly by a sharp blade into cubes about 1 mm. in diameter, the mincing procedure stopped within 15-20 seconds even though not all of the tissue might be finished. The small pieces were then immediately transferred into about 2 ml. of the fixative in a small tube.

Fixation:

Fixation is done in 1% buffered osmium tetroxide. Dalton's buffer (29) was used because it keeps its pH for a long time once adjusted, and does not need frequent checking. A reduction of the extraction of protoplasmic materials over that obtained by other more commonly used fixatives is also claimed for a combination of chrome-osmium fixatives. Sucrose was added to the buffer to increase the tonicity of the fixative and this to avoid

swelling of tissue components. The fixatives must be freshly prepared several hours before its use as follows:-

A. Buffer Stock Solution.

2% potassium dichromate brought to pH 7.2 with potassium hydroxide 1 vol.

1.7% sodium chloride ½ vol.

B. 10% Sucrose Stock Solution in distilled water.

To 5 ml. of the buffer stock solution is added

5 ml. of the sucrose stock solution and

0.1 mgm. osmium tetroxide crystals

The mixture is left in the refrigerator for a minimum period of 4 hours and is used within 48 hours of its preparation.

The small pieces of the tissue are kept in the fixative for 60 minutes, the bottle gently shaken two or three times during the period of fixation.

Dehydration:

Dehydration was then carried out as follows:

15 minutes 10% ethyl alcohol, in the refrigerator

15 minutes 10% ethyl alcohol, room temperature

30 minutes absolute alcohol, room temperature

30 minutes absolute alcohol, room temperature

30 minutes absolute alcohol, room temperature

The tissue then becomes ready for embedding.

Embedding.

Embedding was done in methacrylate and in araldite. The human material was all embedded in methacrylate while the animal material from each experiment was divided in two portions, one half embedded in

methacrylate and the other in araldite.

A. Methacrylate embedding.

A mixture of butyl methacrylate (obtained from I.C.I.) and methyl methacrylate (obtained from B.D.H.) 9:1 was used. This was found to give the optimum hardness of the final blocks.

Methacrylates are supplied as monomers containing hydroquinone as a polymerization inhibitor. This is removed by washing with sodium hydroxide as follows:

A separatory funnel is filled about half full with the methacrylate and about half as much 20% sodium hydroxide in water is added. The mixture is shaken vigorously and then allowed to layer. The sodium hydroxide solution settles to the bottom and becomes brown in colour. After draining off, fresh sodium hydroxide solution is added, shaken and removed. This is repeated until the sodium hydroxide layer is no longer discoloured, indicating that all of the hydroquinone has been removed. The process is repeated using distilled water in place of sodium hydroxide solution to wash out the sodium hydroxide, draining the water layer each time. After draining out the last bit of the water layer, the methacrylate is filtered through three layers of folded filter paper to absorb most of the remaining water. Any water still remaining is removed by shaking the filtered methacrylate with silica gel. Two grams of benzol peroxide are then added to each 100 ml. of the "cleared" methacrylate as a polymerization catalyst. The "catalysed" solutions of methacrylate are then stored in the refrigerator ready for embedding.

To embed in methacrylate the dehydrated tissue is placed in 1:1 absolute alcohol:methacrylate for 3-5 minutes, then transferred to

methacrylate and kept at room temperature for 30 minutes. It is then transferred into another change of methacrylate and kept in the refrigerator until embedded.

Embedding is done in gelatin capsules No. 00. The capsules are filled with methacrylate, lined up in a rack, and placed in an incubation at 56°C for 45 minutes when the methacrylate becomes syrupy in consistency. This prepolymerization has been found to reduce greatly polymerization damage (117) to which tissue embedded in methacrylate is liable. Each cube of tissue is then placed at the bottom of a capsule containing pre-polymerization methacrylate syrup and polymerization is continued by putting the capsules in another incubator at 37°C for two days.

B. Araldite Embedding.

The epoxy resin "Araldite" is an attractive alternative to the methacrylate as an embedding medium for electron microscopy. The setting process occurs uniformly with virtually no shrinkage or damage to the tissue. However, it does not always penetrate the tissues as well and the thin-section-cutting process is rather more difficult for blocks embedded in araldite.

The araldite resin system is prepared from the following materials:

a) Epoxy resin "Araldite" CY 212
b) Hardener 964B
c) Accelerator 964C

obtained from Ciba, C.A.R.L.D., Duxford, Cambridge

d) Di-n-Butyl Phthalate obtained from B.D.H.

Araldite and the hardener are mixed in equal parts in a large jar and stirred with a clean glass rod from time to time and kept in the incubator at 37°C for a few days to ensure complete and thorough mixing.

The dehydrated blocks of tissues are then incubated at 56°C for one hour in a few millilitres of the araldite mixture. The araldite mixture is then replaced with fresh araldite mixture for another one or two hours. The blocks are then impregnated with araldite and can be stored in araldite at room temperature or can be embedded in the gelatin capsules.

For embedding an accelerator-plasticizer is prepared as follows:

Dibutyl phthalate	20 ml.
accelerator 964C	6 ml.

19 ml. of the araldite mixture is then added to 1 ml. of the accelerator-plasticizer mixture in a well stoppered tube and thoroughly mixed in an electric shaker for 12 hours. The blocks are then embedded in this new mixture in the gelatin capsules, placed in the incubator at 56°C for at least three days.

Unlike methacrylate, the hardened resin is not degraded by electron bombardment. Consequently, there is no similar clearing of the sections in the electron beam. On account of the diminished contrast obtained for sections embedded in araldite, staining is usually necessary.

Section cutting:

Ultrathin section cutting was done by -

1) Porter-Blum microtome, and

2) Huxley microtome.

Methacrylate embedded blocks were cut on the Porter-Blum microtome, while araldite embedded blocks were cut on the Huxley microtome.

The Porter-Blum microtome (manufactured by Ivan Sorvall Inc., Norwalk, Connecticut, U.S.A.) has a mechanical advance system consisting of a screw

thread lever arm and suitably designed bearings.

The special specimen by-pass slide, unique to this microtome, provides for cutting alternate thick and thin sections, without interrupting the sectioning procedure or resetting the advance, so that light and electron microscopy may be done on adjacent sections.

The preparation for the cutting of sections consists essentially of trimming the embedded block to the desired face as judged by examining a thick section on the phase contrast microscope, and mounting it in the chuck of the microtome, fitting a knife with a trough for collecting the sections, mounting the knife in the microtome, filling the trough with 20% acetone in distilled water and adjusting the fluid level and illumination, facing the block with the knife, and adjusting the microtome advance to give sections of the desired thickness. Sections are cut while observing the block through a dissecting microscope. After cutting, the thin sections are picked up on electron microscope grids after they have been expanded by exposure to xylol vapours while they are floating on the surface of the trough.

Glass knives cut by the "red-hot-tip" technique were used in the Porter-Blum microtome. Details of the knife preparation and of the technique of thin-section-cutting on the Porter-Blum microtome are found in (117 , 152).

The Huxley microtome (Cambridge) was particularly useful for cutting araldite-embedded material, which are more difficult to cut than methacrylate embedded blocks. This microtome is purely mechanical in principle and its exceptional performance is achieved mainly through the incorporation of several special features. The bar carrying the specimen, and the

lever which advances the bar, are both entirely supported by flexible steel strip or wire connections so that backflash, friction, wear and lubrication difficulties are completely avoided. The main feature of this microtome is the fact that gravity is used to advance the specimen holder for the cutting stroke. The operating arm is lifted by hand between successive cuts and allowed to fall under its own weight for the cutting stroke. The speed of fall is controlled by a plunger in an oil-filled dashpot. A more uniform row of thin sections are obtained by the use of the Huxley microtome as compared with the Porter-Blum, and usually a bigger block face can be cut on the Huxley machine. However, it is rather slow to get through the block on the Huxley microtome and the block cannot be trimmed in situ but has to be taken out of the chuck for that purpose. To avoid these two difficulties araldite blocks were first mounted on the Porter-Blum microtome and thick sections cut until the desired piece of tissue is found. The block is then trimmed to that face on the Porter-Blum machine and then transferred to the Huxley microtome for the final process of thin-section-cutting.

For the Huxley microtome, glass knives were cut by using a jig, tungsten carbride wheel and two pairs of glaziers' pliers to obtain knives of an exact size and shape to fit the knife holder in this machine. When cutting araldite blocks, the knife trough was filled with distilled water rather than an acetone solution for floating the sections. Details of the use of the Huxley microtome were obtained from (157).

Specimen Grids:

The copper supporting grids used for thin sections were obtained from "Athene" Specimen Grids, Smethurst, Highlight Ltd., Bolton, Lancs, England.

Two types of grids 3.05 mm. in diameter were used; "Type new 200" for the methacrylate sections and "Type 483" for the araldite sections. The "new 200" grids were coated with a thin film of collodion and another thin film of carbon before mounting the methacrylate sections. The araldite sections were picked up directly on "483". grids without any supporting film. This could be done since the "483" grids have much smaller holes. This was necessary with the araldite sections because the araldite itself is rather electron dense and the tissue had to be stained to increase the contrast; during the process of staining solvents are used which dissolve any thin film of plastic material on which the sections might be mounted.

Thin films of collodion were prepared by placing about 30 "new 200" grids on a small tray of fine-mesh wire gauze placed in the centre of a large deep dish filled with water to its brim. A drop of 3% collodion solution in amyl acetate is then put on the surface of the water where a thin film of collodion would immediately form. Water is then sucked very slowly by a suction pump from the dish and the film is allowed to come down very slowly over the grids. The grids are then kept in a dessicator for at least 24 hours before a carbon film is put on.

Sections mounted on plastic films have a definite tendency to drift in the electron beam, making photographic recording of the image difficult. The use of carbon support films almost entirely overcomes this difficulty. (160, 161). In fact the stability of sections mounted on carbon films is such that photographs may be made as soon as focusing has been completed and the intensity adjusted to the proper level, without any special attention being paid to waiting for drift to cease. Carbon films are also stronger than plastic films and do not tear as readily in the beam.

To make carbon grids, at least 24 hours after the collodion coated grids have been kept in the dessicator, they are placed in a vacuum evaporator about 15-20 cm. away from a carbon arc. The carbon rods forming the arc should be spectroscopic grade graphite and are sharpened to points of 1 mm. diameter or less. One of the carbons should be in a sliding holder and loaded with a spring or weight and lever arrangement to keep contact with the arc. The chamber is evacuated to 0.05 μ Hg. pressure or less, and alternating current sufficient to cause sparking of the arc and evaporation of carbon is passed through the carbons. The current should be somewhat greater than the minimum required to produce sparking. About 30-40 amperes is usually required. The current is shut off when sufficient carbon has been evaporated onto the grids, which requires only a few seconds. The amount of carbon to evaporate can be determined with a detector consisting of a piece of white porcelain about 1 cm. square with a small drop of diffusion pump oil in the centre. This is placed next to the grids, and turns tan to brown when the carbon is deposited, the oil spot remaining white and serving for comparison. A very light tan colour indicates a carbon film of the proper thickness.

"Staining" for electron microscopy.

Osmium tetroxide has very important staining properties associated with its qualities as a fixative. Fortunately, osmium tetroxide seems to combine in such a variety of ways with many things that it quite effectively serves as a general purpose stain. Practically all the methacrylate embedded material studied in this thesis were only fixed (and simultaneously "stained") with osmium tetroxide.

In order to "stain" structures that are not particularly affected by

osmium tetroxide, and to boost the contrast of those which are, all the araldite embedded sections, which have a poor contrast of their own, were "stained".

Two "stains" were used -

1) PTA: 1% solution of phosphotungstic acid in 50% alcohol. The grid on which the sections are mounted is dipped in the "stain" for one minute then washed by dipping in clear 50% alcohol for a moment. However, this stain was found to increase electron density tremendously and to give rise to some granular deposits.

2) Lead acetate: A supersaturated solution of lead acetate in ether: ethanol 1:1 is used. The grids are placed for 10 minutes in the stain, then immersed for 10 minutes in absolute ethanol for washing. This was found to give perfect staining particularly for the cell membrane.

Microscopy.

All the studies were done by an E.M.6 electron microscope (AEI). This type of electron microscope is a high resolution instrument of a new design embracing recent advances in electron optics, with a variety of features contributing towards results of the highest qualities. Features especially worthy of note are the objective lens, which embodies a fully adjustable mutliple aperture system; the double condenser which gives complete control of illuminating spot size; and the independently pumped airlocks for the specimen chamber, and the recording camera. A resolving power of 15 angstrom units is guaranteed by this microscope.

The accelerating voltage used in most of the studies is 50 KV, providing electron beam-wavelengths of 0.05 Å. Occasionally an accelerating voltage of 75 KV was used.

The recording camera is a multiplate automatic camera and the electron micrographs were taken on $3\frac{1}{4}$ x $3\frac{1}{4}$ inch plates.

THE NORMAL GLOMERULUS.

THE NORMAL GLOMERULUS.

Delineation of the normal structure of the renal glomerulus is a requisite as a base line for evaluation of pathological alterations. Glomeruli from three mammalian species were studied by the electron microscope: human, rabbit and rat. Two strains of rats were studied, "Wister" albino rats and "Sprague-Dawley" hooded rats. Except for a slight variation in the thickness of the basement membrane, the ultrastructure of the renal glomerulus was found to be essentially the same in all three species.

My findings confirm the well known structural features of the glomerular capillary wall: namely the existence of a continuous basement membrane, lined on the luminal side by an extensively fenestrated endothelium and covered towards the urinary spaces by a continuous array of interdigitating epithelial foot processes (Fig. 1). The endothelial cells are three to four times as numerous as the epithelial cells, and their nuclei are somewhat smaller and denser than those of the latter (Fig. 2). Endothelial cytoplasm is abundant in the region of the nucleus and contains the usual cytoplasmic components; mitochondria (Fig. 3) a few canaliculi of the endoplasmic reticulum with the associated RNP granuoles, small piles of Golgi cisternae and small vesicles (Fig. 4). At a greater distance from the cell body the cytoplasm becomes attenuated and contains a series of openings, the fenestrae (Fig. 5). The thickness of the attenuated part of the endothelial cytoplasm is irregular; it is quite thin in

the peripheral capillaries, measuring down to 500 Å (cytoplasm and both cell membranes) in thickness. Some sections reveal branching processes of the attenuated endothelial cytoplasm (Fig. 6) particularly in the central capillaries (Fig. 7) with no fenestration. The size and shape of the fenestrae is frequently,but not always,uniform. In cross sections they are seen as repeated interruptions, giving the endothelial cytoplasm a beaded appearance (Figs. 1 and 5). In grazing sections (Figs. 8 and 9) the attenuated layer appears as a sheet with circular or oval openings, 400-1000 Å in diameter separated from one another by cytoplasmic strands 400-1000 Å in width.

In theperipheral parts of the loops where two endothelial cells come together they typically overlap to some degree (Fig. 10). Along their line of contact there are areas of increased density of the opposed membranes (Fig. 11). These specialised regions are similar to the so-called "adhesion plates" (44)which are present along the surfaces of contact of epithelial cells and identical with the "attachment belts" described in vascular endothelia (164). They have been first described by Farquhar and her colleagues in 1961 (44) and have been confirmed in the present study. In addition in the region of the endothelial attachment belt oblique sections show several cytoplasmic processes in a complex overlapping interdigitating manner, indicating that peripherally the endothelial cell cytoplasm is branched and arranged in such a way that the cells resemble starfish with a number of arms resting on the basement membrane,those of one cell interdigitating with those of the neighbouring cells (Fig. 10). When the attachment is more closely examined in cross sections, it can be seen (Fig. 11) that the attenuated endothelial cytoplasm becomes thickened and loses its fenestration as it

approaches the adjacent cell. The attachment belt appears to be particularly strengthened by further thickening of the adjacent cell membranes and the immediately subjacent cytoplasm. Lipping and further thickening of adjacent cell margins on the luminal side completely plugs the very narrow slanting slit (40 Å) between the endothelial cells.

In some electron micrographs, strands of basement membrane-like material are clearly seen within the endothelial cell-body cytoplasm (Fig. 3 and 12) and in places, the endothelial cytoplasm appears to merge imperceptibly with the basement membrane, while the demarcation between the epithelium and the basement membrane is usually distinct (Fig. 13).

Directly adjoining the endothelium is the basement membrane. At low magnification it appears as a homogeneous layer possessing a moderate electron density. At high magnifications there is an indication of a lamellated structure that gives the impression of a spongy system, which is more evident after staining.

The thickness of the basement membrane varies from loop to loop, apparently with the degree of dilation of the capillaries. It is thinner in the peripheral than in thecentral capillaries. Its thickness was found to vary in the different species studied; it was thinnest in the rabbit (Fig. 14) and thickest in the human glomerulus (Fig. 15) while in the rat its thickness occupied an intermediate position (Fig. 16), Table 2.

Table 2.

Species.	Thickness of the glomerular basement membrane.
Rabbit	600 - 1000 Å
Albino Rats	1000 - 1500 Å
Hooded Rats	1000 - 1500 Å
Human	2000 - 2500 Å

Though thin strata of low density were sometimes found on either side of these basement membrane (Fig. 17) the lamina rara externa and the lamina rara interna of various authors, I do not believe that they constitute an integral part of the basement membrane. The width of these lighter areas was found to vary noticeably with the degree of perfection of the section; in perfect preparations the basement membrane nearly fills the space between the endothelium and epithelium (Fig. 18) and I tend to agree with Farquhar and her co-workers (44) that these thin strata are artefacts of extraction incurred during fixation and embedding particularly so, because they were hardly ever seen in araldite embedded material which greatly supercedes methacrylate as an embedding medium free of artefact production.

The outer layer of the glomerular capillary wall is the epithelium. The epithelial cells possess relatively large ovoid nuclei, less dense than those of the endothelial cells and deeply indented (Fig. 19). The nuclei are surrounded by abundant cytoplasm which has a rather light background density and contains scattered formed elements, mitochondria vesicles (Fig. 20), Golgi material (Fig. 21, 20, 16) and a few canaliculi of the endoplasmic reticulum (Fig. 20) and the associated RNP granuoles. Occasionally, large vesicles are seen in the epithelial cytoplasm (Fig. 22) The cytoplasm of the epithelial cells is divided into a number of primary branches, the "trabeculae"; each branch in turn is divided into a number of secondary branches, the "foot processes" or "pedicels" (Fig. 12 and 23). The cytoplasm of the trabeculae is similar to that which surrounds the nucleus and contains RNP granuoles, mitochondria and vesicles. The trabeculae penetrate deeply in the crevices between adjacent capillaries and supply the surfaces with pedicels (Fig. 12). In some electron photomicrographs the great length of these trabeculae was quite

apparent (Fig. 24). Trabeculae may not only radiate from the cell bodies peripherally but also arise from the under surface of the epithelial cells (Fig. 25).

From either side of the trabeculae, a number of foot processes emerge and are applied to the outer aspect of the basement membrane. The contact of a cell body of a podocyte with the basement membrane is substantially through these pedicels. Beneath the podocyte there is a subpodocytic space. Also, in many electron photomicrographs a trabecula appears to overlie some of the pedicels giving a labyrinthine appearance, the so-called pericapillary sinus. (Fig. 14). Pedicels from neighbouring trabeculae of the same or adjacent epithelial cells interdigitate with each other and this is best shown in the tangential sections (Fig. 8). The entire basement membrane is covered by the interdigitating pennate processes of the epithelial cells, which are thus, themselves elevated (Fig. 25).

In the foot processes the cytoplasmic matrix is frequently denser than in the rest of the epithelial cell. As a rule these processes do not contain significant numbers of formed elements except for numerous small vesicles of the type present in large numbers throughout the epithelial cytoplasm.

Each foot process typically has a narrow stalk at the point of origin from its trabecula but expands into a broader base in contact with the basement membrane resembling an elephant's foot (Fig. 14, 17,18 and 26). As a result of this arrangement, the spaces between the foot processes forms narrow slits 150-400 Å in width (mean 250 Å) near the basement membrane and rapidly increases in width towards the urinary spaces. Occasionally a tiny invagination of the cell membrane at the base of a

foot process is seen (Fig. 18, 27) and apparently represents a process of pinocytosis by the pedicel, as suggested by Farquhar et al (44).

In sections normal to the capillary wall and perpendicular to the base of the foot processes, a thin line with ill defined limits is frequently seen bridging the narrowest point of the gap between foot processes (Fig. 8,17,18,23). This line, which has been called byYameda (164), the "filtration slit membrane" has been described only by a few glomerular electron microscopists (44, 116) but its existence has been confirmed in this study. It is thinner and more tenuous than the cell membrane (Fig. 8). In locations where it is seen,there is a thickening of the opposed cell membranes of the foot processes backed by increased density in the slit pore. In a few slit pores two membranes have been seen (Fig. 8).

As Noted by most previous workers, the nuclei and cell bodies of endothelial cells are usually located in the axial or deeper parts of the loops (Fig. 2,28). In these regions the cells are frequently grouped and show some piling or stratification (Fig. 29). The superficial cells can be clearly seen to line the capillary lumen, but the exact relationship of the deeper cells to the lumen is not always evident. Sometimes these deeper cells show rounded pseudopodia which push through the cytoplasm of the superficial cells to establish contact with the lumen. The surface of the basement membrane is irregular in the axial regions and usually appears to be provided with spurs or processes of varied form which penetrate between the endothelial cells (Fig. 30). Sometimes, in the plane of sectioning a mass of tissue composed of cells with (Fig. 31) or without (Fig. 32) nuclei and projections of cytoplasm is seen separated from the

capillary lumen by endothelial cytoplasm and from Bowman's space by a branch of the capillary loop basement membrane, on which rested epithelial pedicels. In electron photomicrographs taken in such a way as to include most of a lobule in cross-section (Fig. 28) the basement membrane does not surround individual capillaries, but encircles the entire lobule in a continuous fashion. It dips deeply between the adjacent capillaries but does not separate them completely: instead, it reflects across the axial region to the next capillary. Thus, the basement membrane includes two to eight capillaries which are in turn distributed in an irregular "clover-leaf" fashion around a central cytoplasmic mass with the basement membrane following the outer contour of the complex. Epithelial foot processes are applied to the outer surface of the membrane throughout its extent, while within it are endothelial cells and cells occupying the axial region.

The above description appears to confirm the view that in the glomerular tuft a third type of cell, which lies singly or in groups in the centre, does exist. However, when the matter is more closely studied considerable doubt is cast over the existence of such a third type of cell. Those workers who recognised a third type of cell in the glomerulus mainly depended upon seeing cells that do not contribute to the lining of a capillary lumen in a given plane of section as those seen in Fig. 30,31,32,33). It is quite conceivable that such a cell might in fact do so above or below the plane of section. On the basis of electron micrographs of single sections, the existence of a third type of cell and the denial that such cells are tangentially sectioned endothelium (and even epithelium) cannot be founded.

Some electron microscopists (76, 153, 164) who believe in the presence

of a third type of cell, have described morphological differences from endothelial cells e.g. in the density of their cytoplasm, and considered such a difference in morphology a further evidence supporting their existence. However, most electron microscopists, including some of those who believe in their existance, as a separate type of cell (109, 136) have failed to find clear-cut cytologic features which would facilitate their recognition. Certainly, I have found no morphological difference between the axial cells and the usual endothelial cells, and in reviewing the published electron photomicrographs of those authors who describe the presence of characteristic features in these cells, I could not satisfy myself to the presence of such features.

The principal problem is one of three dimensional interpretation of thin sections. There is no doubt that a capillary may be cut in such a fashion that an endothelial cell body appears to fill the lumen or bridge across it, creating the appearance of two or more lumina separated by a cell mass. When the main body of endothelial cytoplasm (and the nucleus) is situated at a capillary bend, a section through it, at right angles, would exhibit the projection of endothelial cytoplasm away from contact of the lumen and can be wrongly interpreted as intercapillary (Fig. 30,32, 34,35,36,37).

In the middle of what appears as intercapillary tissue, obvious epithelial pedicels are sometimes seen in electron micrographs (Fig. 38, 39,40). Pedicels in this position are the result of deep penetration from the surface in the crevice between two capillaries (Fig. 41,42). If an epithelial cell (Fig. 43) or a segment of a trabecula (Fig. 41,42) is directly applied to the surfaces of two adjacent capillaries without pedicel formation, as occasionally happens, a cross-section at right angles

in this area will produce the appearance of a lake of cytoplasm with or without a nucleus, entangled between two basement membranes and might superficially be regarded as an intercapillary cell, as its epithelial character will then be beyond recognition.

I have been able to confirm, to my satisfaction, by means of few serial sections, that most of the cells embedded in the basement membrane-like masses are,in fact, capillary endothelial cells of which the part enclosing the lumen is in another plane of section. However, the end of the long controversy, as to whether these intercapillary cells do in fact exist or do not, will be finally established only by means of uninterrupted serial sections for electron microscopy, something that has not yet been reported.

The glomerular tuft is covered by Bowman's capsule consisting of a thin basement membrane lined by flat epithelial cells. The basement membrane of Bowman's capsule is slightly thicker than that of the glomerular capillaries, measuring between 1500-3000 Å (Fig. 44,2,5, 28). In structure it appears much more definitely fibrillar than the glomerular basement membrane. The lining cells are simple flat squamous epithelial cells with much fewer cell organelles than the visceral epithelial cells covering the glomerular capillaries.

The Glomerular Arterioles.

The glomerular arterioles were similar in structure in the three species studied. The arteriole is lined by endothelial cells that lie on an internal elastic lamina which separates the endothelium inside from the smooth muscle cells outside (Fig. 47). The endothelial cells join in the same ways as in capillaries, i.e. by apposing or overlapping (Fig.48,49,50).

Their plasma membranes show indentations and vesicles to a varying degree. (Fig. 50). The comparatively dense cytoplasmic layer may be reduced to 200 mμ (Fig. 49,50,51). The internal elastic lamina consists of materials of different densities; in many places the more opaque forms an outer layer. It is frequently fenestrated and protrusions of the endothelial cells contact the smooth muscle cells through the windows (Fig. 48). It seems likely that each endothelial cell possesses such protrusions. The material of the internal elastic lamina continues without sharp border line between adjacent smooth muscle cells (Fig. 49,50). Small invaginations and vesicles, as seen in the endothelial cells (Fig. 51) are also observable on the plasma membranes and in the cytoplasm respectively of these cells (Fig. 51). The contractile material of the smooth muscle cells seems to consist of extremely fine filaments. Small mitochondria are located predominantly in the central areas of the cells (Fig. 47,48). Connective tissue fibrils occur in the adventitia.

The arteries.

Interlobar, arcuate and interlobular arteries were examined; they showed the classical description of arteries of comparable size elsewhere in the body (Fig. 52, 53).

The Juxtaglomerular apparatus.

The juxtaglomerular apparatus was studied in the normal rat and in one albino rat that has been forcibly hydrated previously. It was found to consist of three elements:

1) Modified muscle cells.

2) The macula densa.

3) Ground substance network.

As the "afferent arteriole" approaches the glomerulus its structure greatly changes. A group of cells become apparent in the adventitia and the muscle coat in place of the usual smooth muscle cells. They are slightly larger in size,(compare Fig. 54 with Fig. 47) plump and elongated and have a small nucleus. Their cytoplasm looks loose with small rounded mitochondria apparently crowded around both poles of the nucleus. A definite pile of Golgi cisternae can be seen close to one pole of the nucleus (Fig. 55). In the normal rat, these cells showed some strongly osmiophilic granules delineated by a single membrane, while in the hydrated rat they completely lacked these granules. (Fig. 54). The cells are present in a compact cell mass but individual cells are not in contact with each other; an intricate system of canals runs in between.

The macula densa is a group of cells along the course of the distal tubule as it comes in contact with its glomerulus and its appertaining afferent arteriole. Its cells differ from the adjoining epithelial cells of the distal tubule, whether they are the preceeding thick segment of the ascending limb of the loop of Henle, or the following convoluted part of the distal tubule. They are smaller in size and have fewer mitochondria. The mitochondria are shorter and lack orientation; they are not longitudinally arranged in the basal region but are disseminated throughout the cytoplasm. The most peculiar characteristic that they possess, however, is the presence of very abundant cytoplasmic membranes which traverse across the cell and freely anastomose with the ground substance network.

Between the macula densa, the glomerulus and the afferent arteriole is found a group of cells best termed "the network cells" or the "lacework cells". They have small rounded nuclei with fine chromatin dots and a

small nucleolus surrounded by a small rim of cytoplasm. The greater part of the cytoplasm is immediately separated into elongated fine ramifications occupying the interstices of a lacework formed by prominent membranes interwoven into a structure of an extreme degree of complexity (Fig. 56, 57). These membranes appear similar to the complexly folded basal cell membrane of the renal tubular cells. However, they differ from cell membranes by the fact that they are single-layered, and not triple-layered, in this character resembling the endoplasmic reticulum. The cytoplasmic extensions that can be recognised amidst this complex network is a relatively clear cytoplasm with few RNP granules occasionally seen around small vesicles. Few mitochondria, vesicles and Golgi cisternae can sometimes be seen. The lacework is continuous with the basal cell and cytoplasmic membranes of the macula densa on one side and with the system of canals running in between the modified muscle cells of the afferent arteriole on the other side,without the interposition of basement membranes or capillaries between these structures.

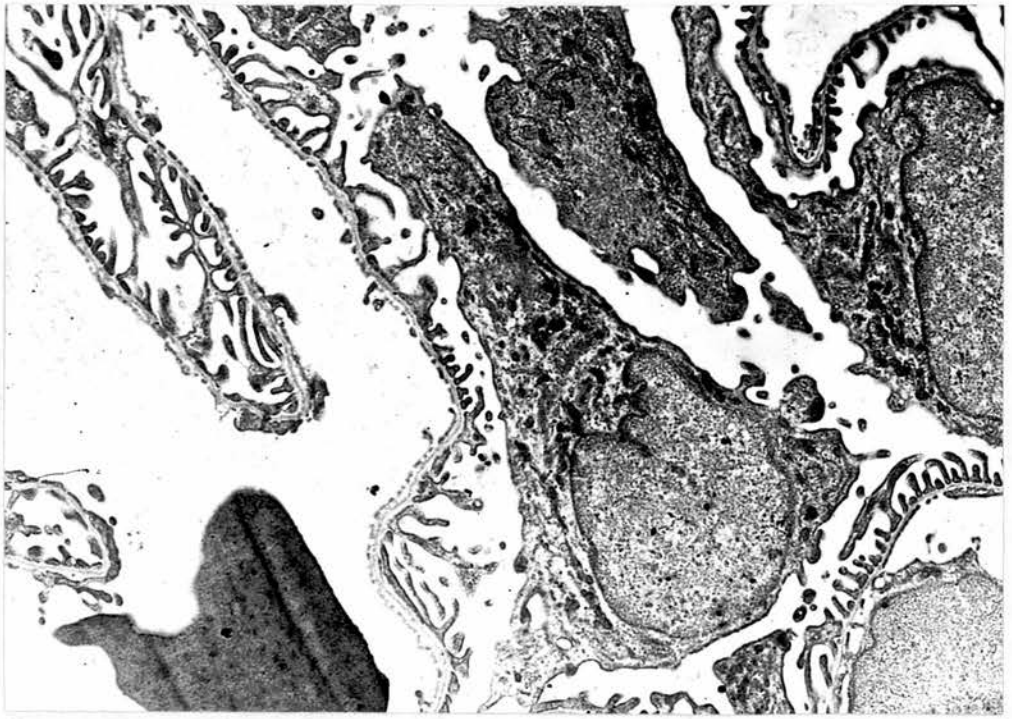


Fig. 1. Three glomerular capillaries from the kidney of a normal adult rabbit. The capillary wall consists of a basement membrane lined by an attenuated fenestrated endothelium and covered by a continuous array of interdigitating foot processes. An epithelial cell is seen in the centre of the field. x 6,000

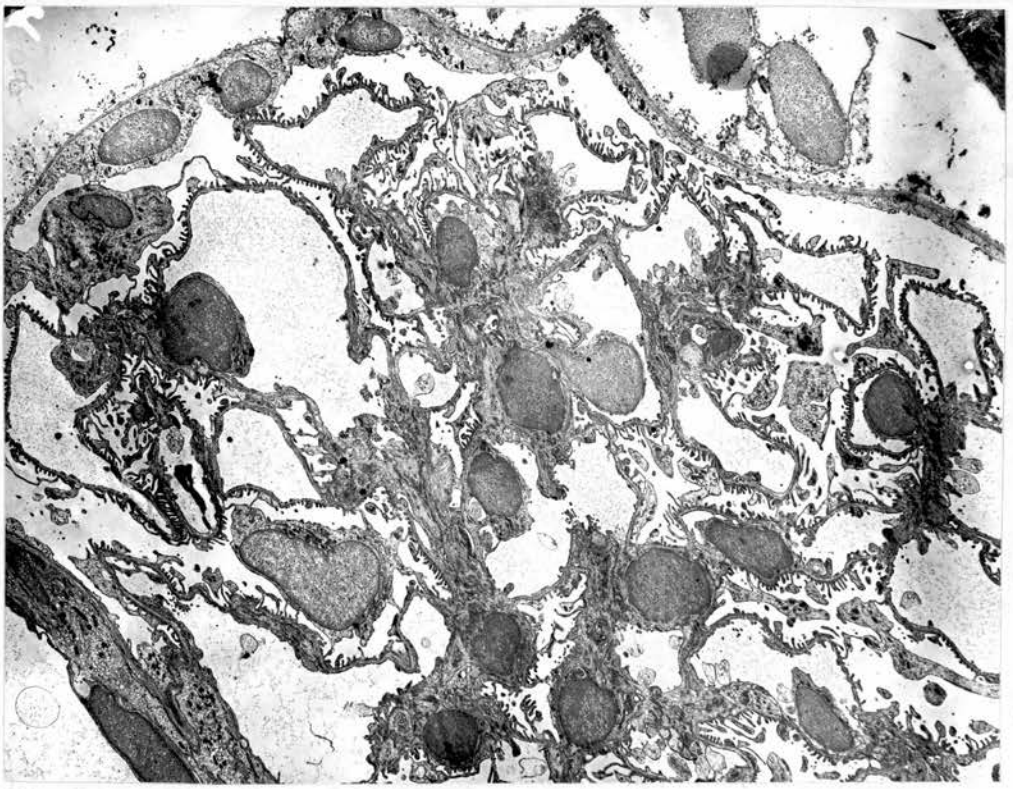


Fig. 2. A low power electron micrograph showing the general architecture of the glomerulus from a normal adult rabbit. Twelve endothelial, but only two epithelial cells can be seen. The endothelial nuclei are smaller and slightly denser than the epithelial nucleus. x1,200

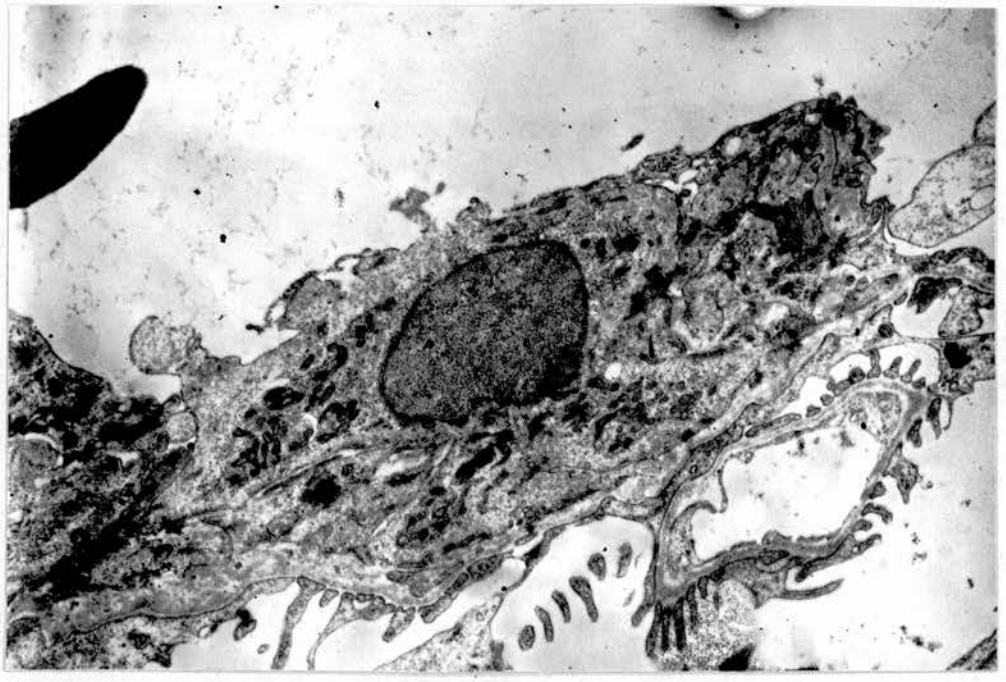


Fig. 3. Glomerular endothelial cells from a rabbit's kidney. The cytoplasm is abundant around the nucleus and contains a moderate amount of small mitochondria. x 12,000

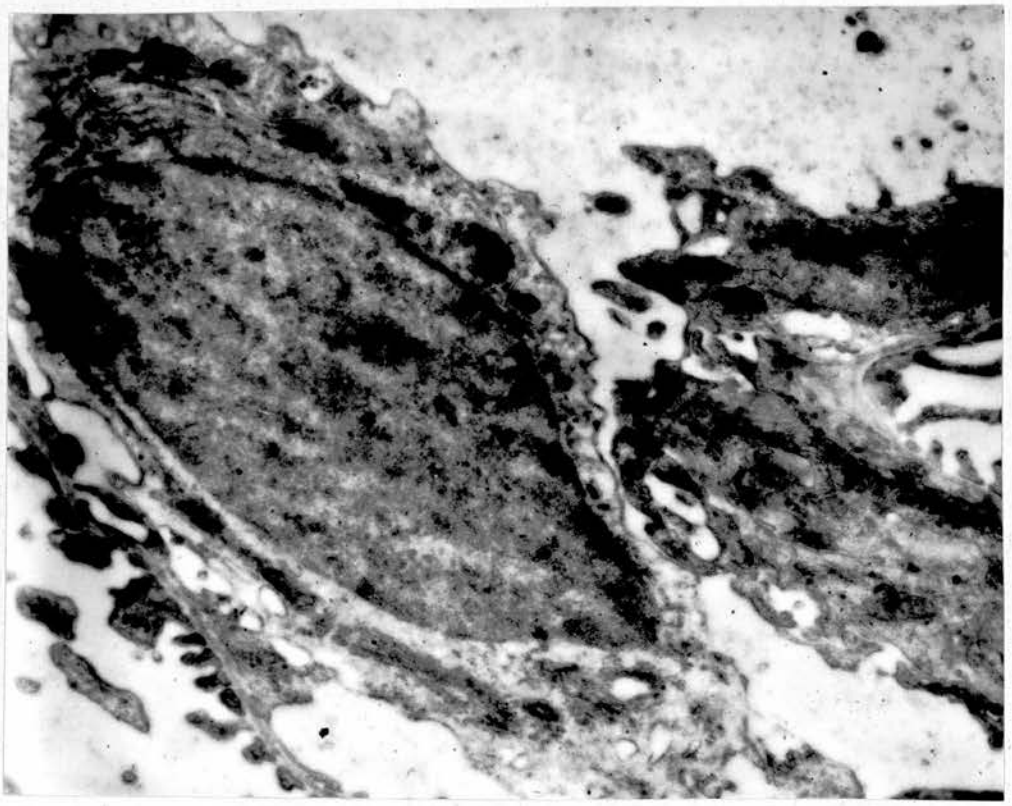


Fig. 4. Glomerular endothelial cell from a hooded rat's kidney. Note the pile of Golgi cisternae at the upper pole of the nucleus and the cytoplasmic vacuoles at its lowerpole. x 15,000

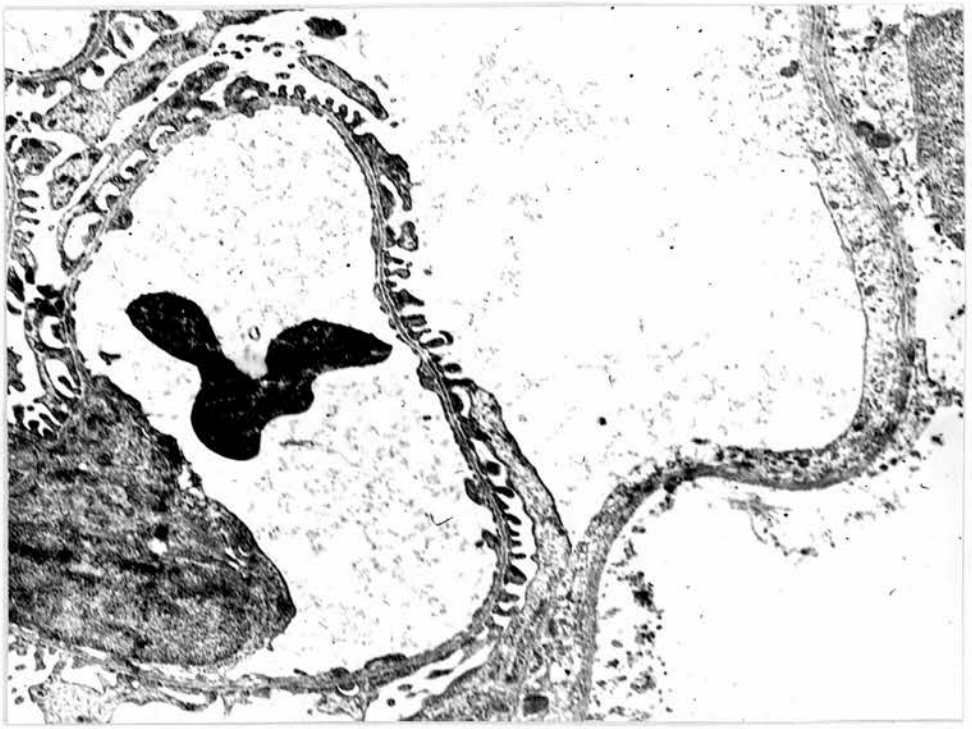


Fig. 5. Glomerular capillary from a hooded rat's kidney. Note that the endothelial cytoplasm, which is abundant around the nucleus, becomes very attenuated and fenestrated in the peripheral part of the capillary. x 6,000

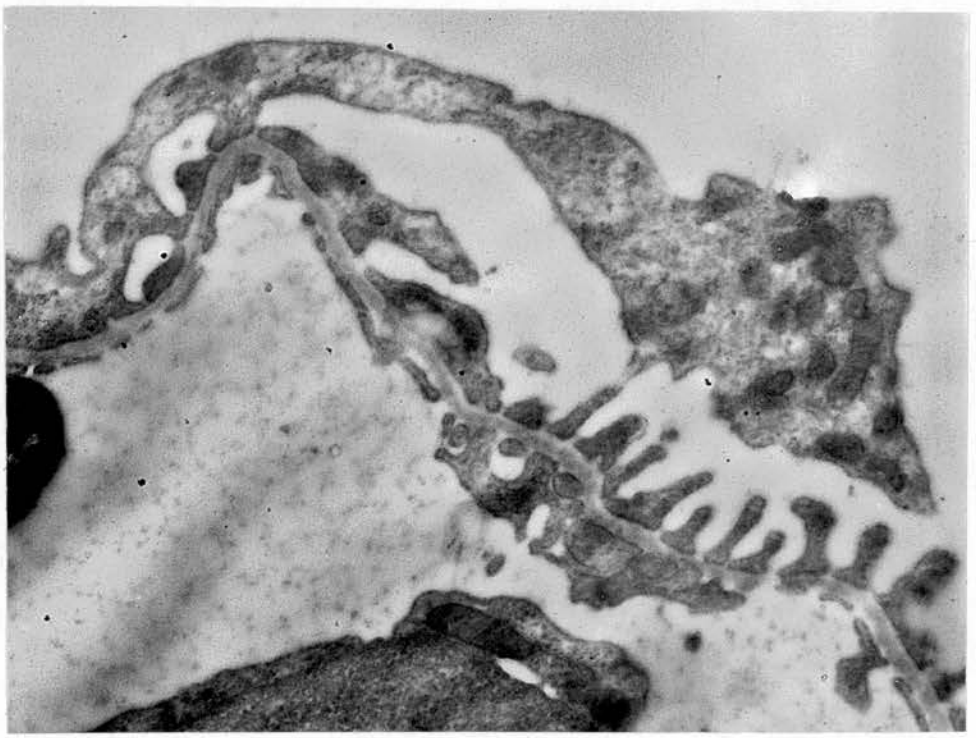


Fig. 6. Glomerular capillary wall from a hooded rat. Note the branching processes of the attenuated endothelial cytoplasm and the long epithelial trabecula. x 24,000

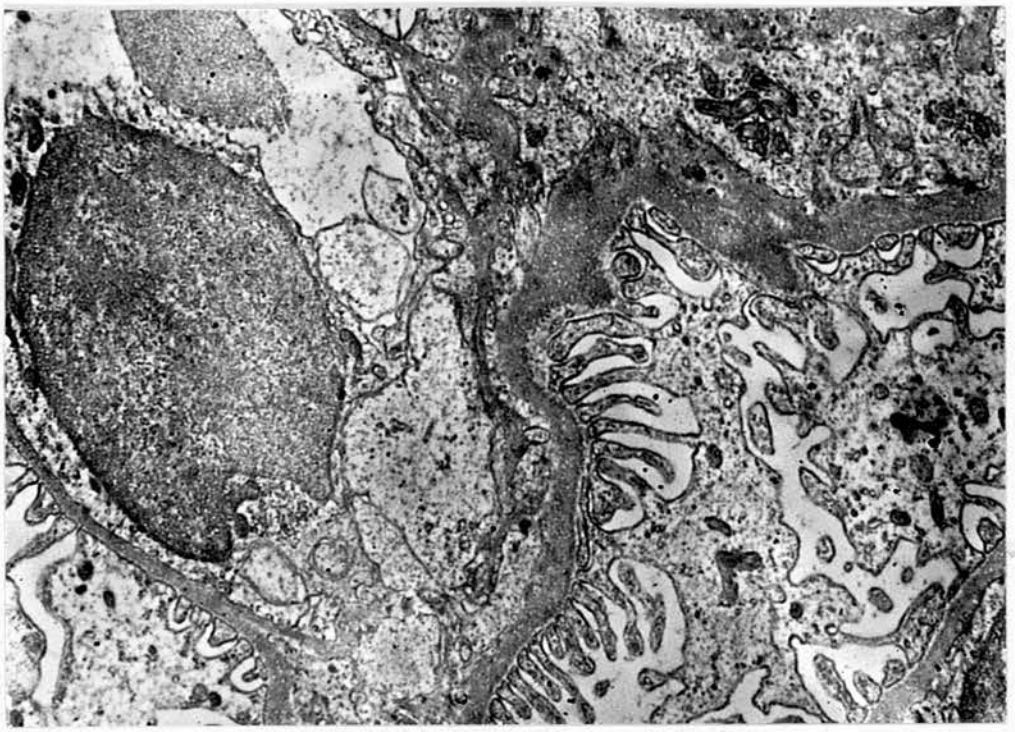


Fig. 7. Glomerular capillary from a normal adult albino rat. Note the branching processes of the endothelial cell.
x 12,000

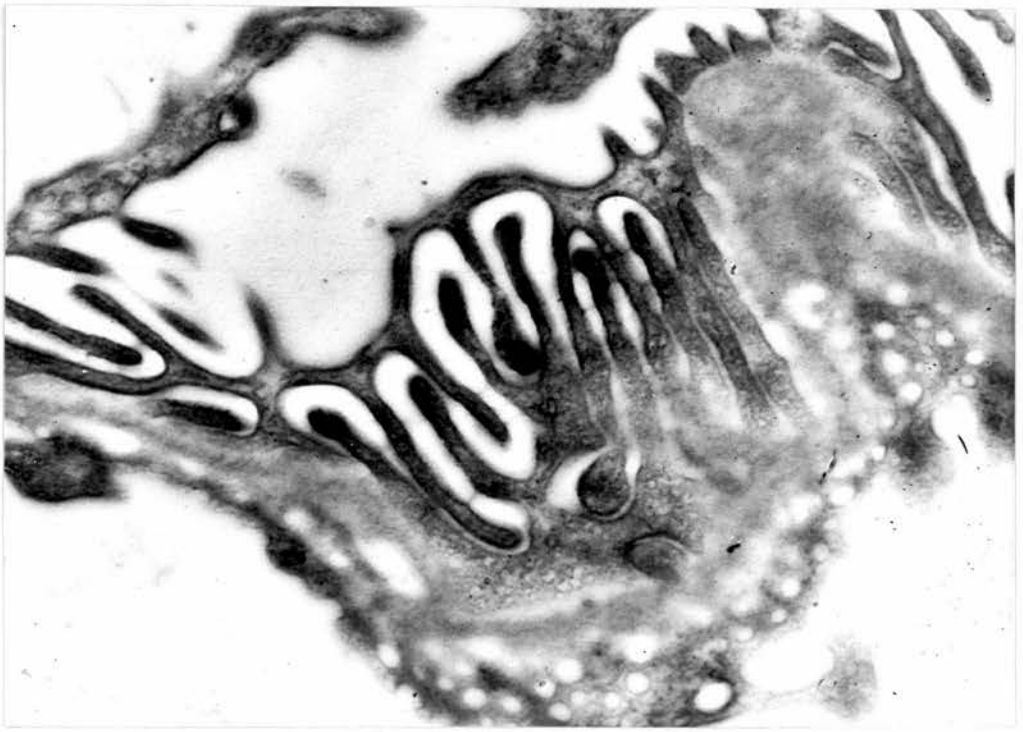


Fig. 8. A grazing section of a glomerular capillary wall from a rabbit's kidney. The endothelial fenestrae appear as rounded or oval holes. Note the filtration slit-membrane; two membranes can be seen connecting two adjacent pedicels.
x 30,000

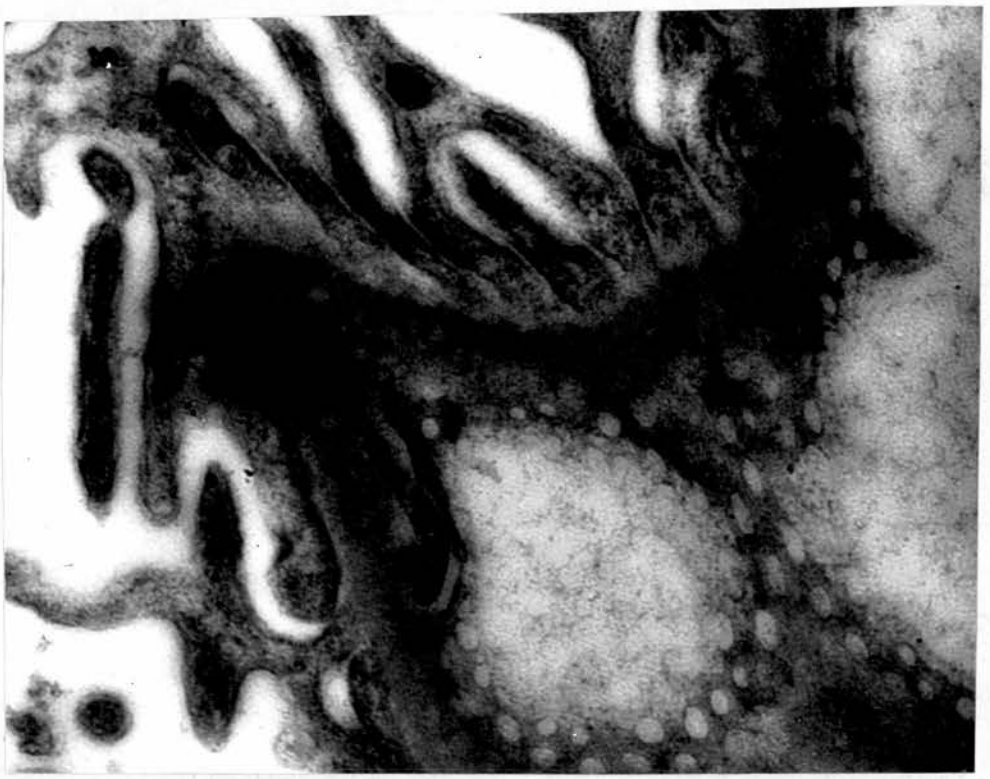


Fig. 9. Glomerular capillary wall from an albino rat. The fenestrae in the attenuated part of the endothelium look like a chicken fence in this tangential section. x 45,000

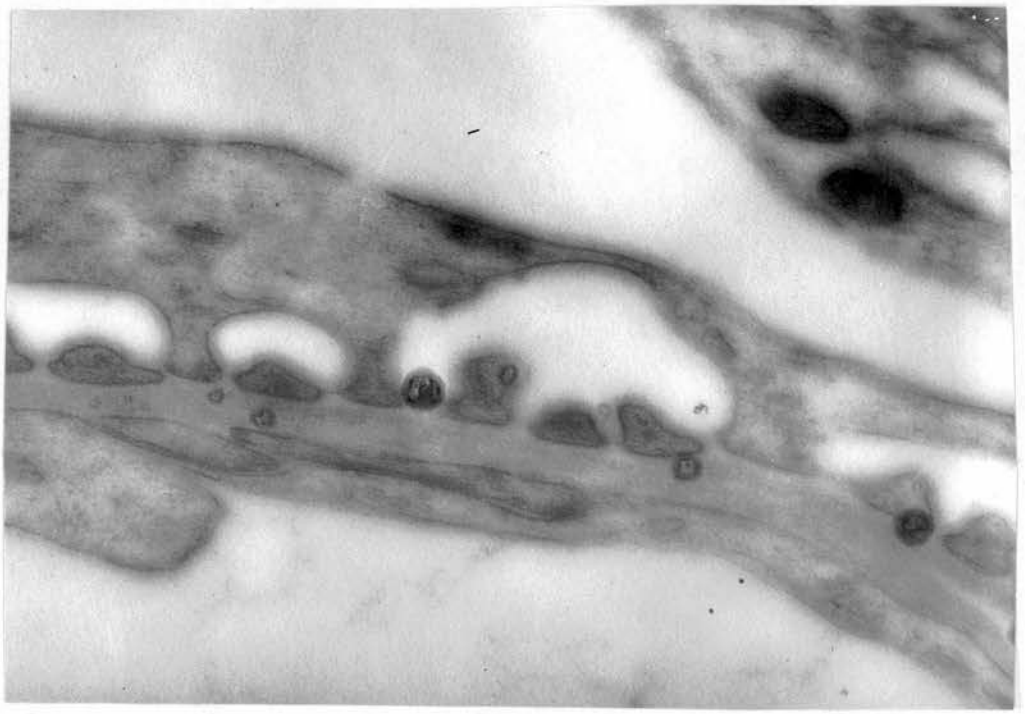


Fig. 10. Glomerular capillary wall from an adult rabbit. Note the filtration slit-membrane and the endothelial attachment belt. x 60,000

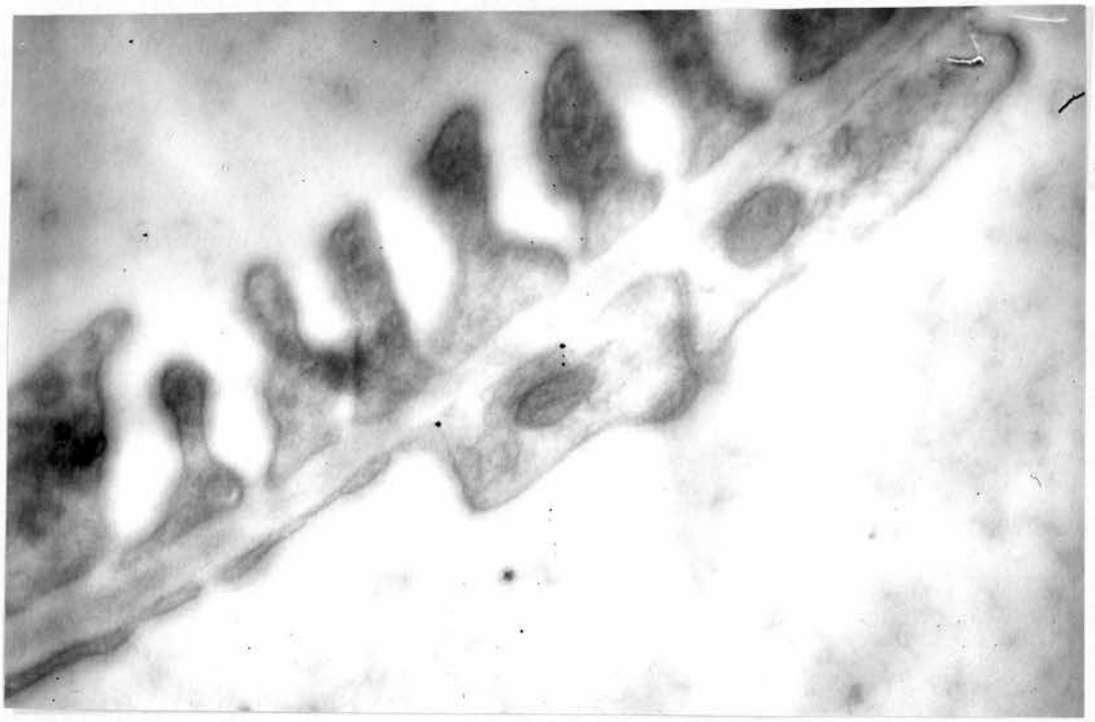


Fig. 11. Glomerular capillary wall from a hooded rat. Note the endothelial attachment belt. x 60,000

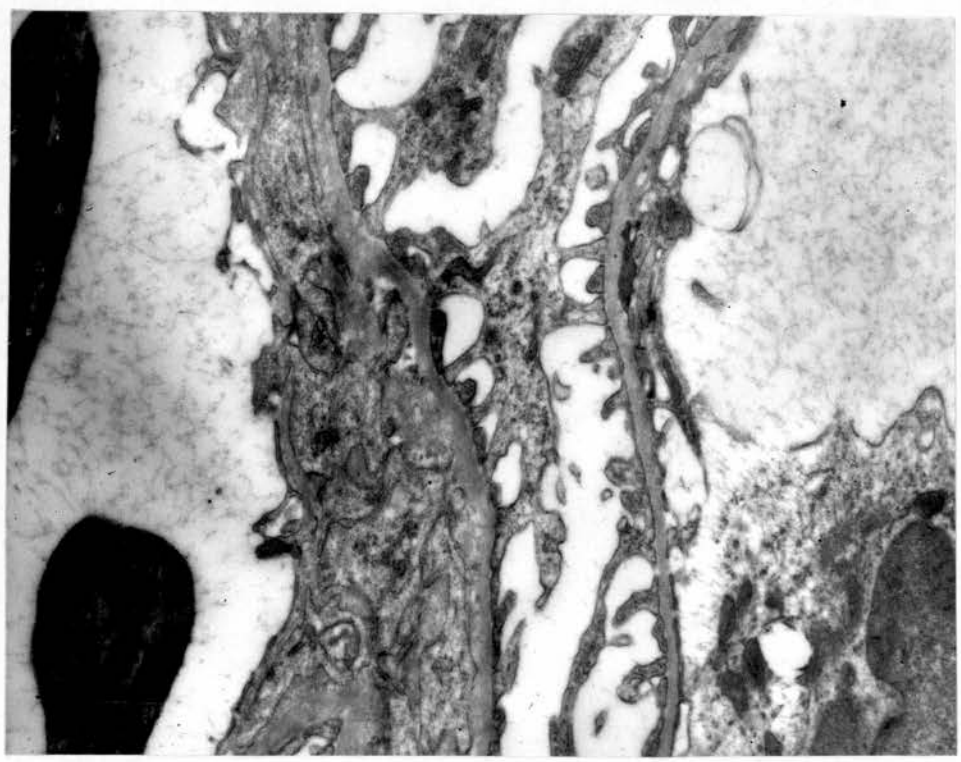


Fig. 12. Two adjacent glomerular capillaries from an albino rat. Note the strands of basement membrane intermingled with the endothelial cytoplasm in the capillary on the left.
x 15,000

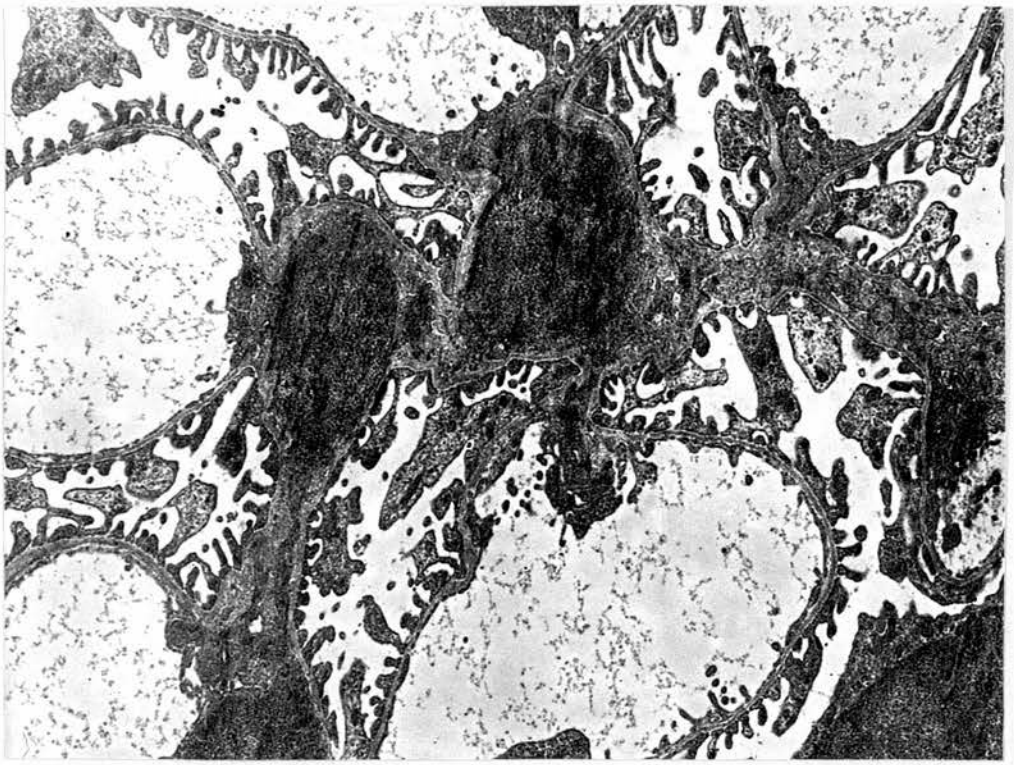


Fig. 13. Glomerulus from a hooded rat. The endothelium appears to merge imperceptibly with the basement membrane, while the demarcation between the basement membrane and the epithelium is distinct. x 6,000

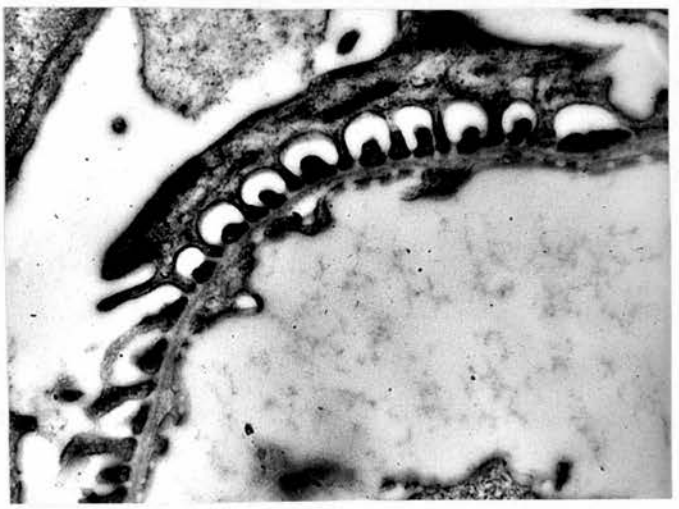


Fig. 14. Capillary wall from a normal rabbit's glomerulus. Note the thickness of the basement membrane and compare with Fig. 15 & 16. x 16,000.

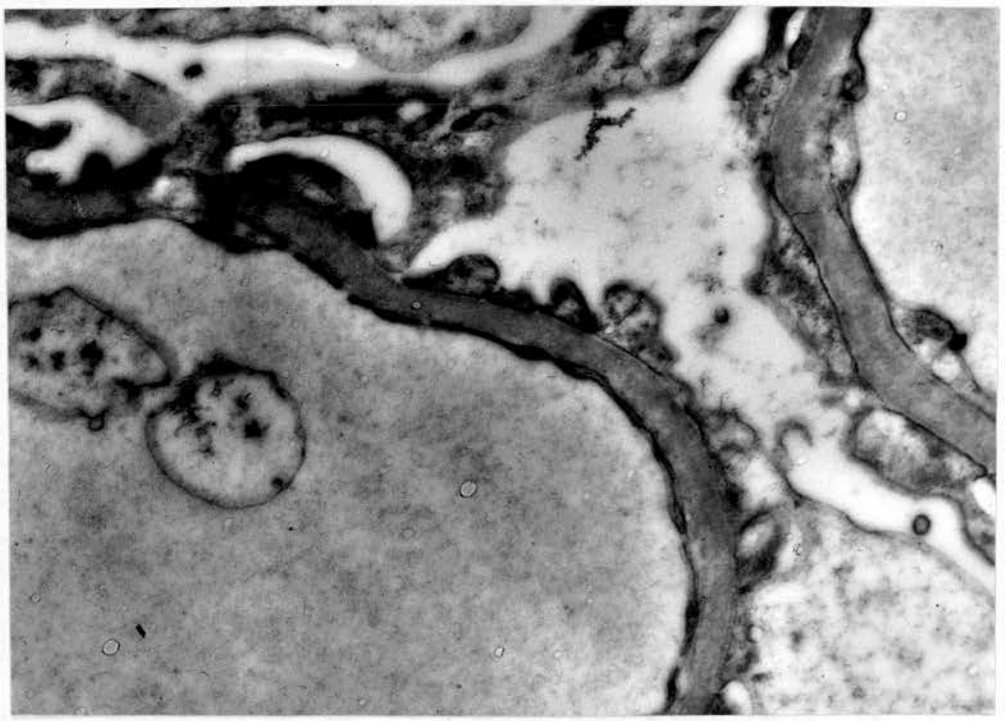


Fig. 15. Capillary wall from a normal human glomerulus.
x 16,000

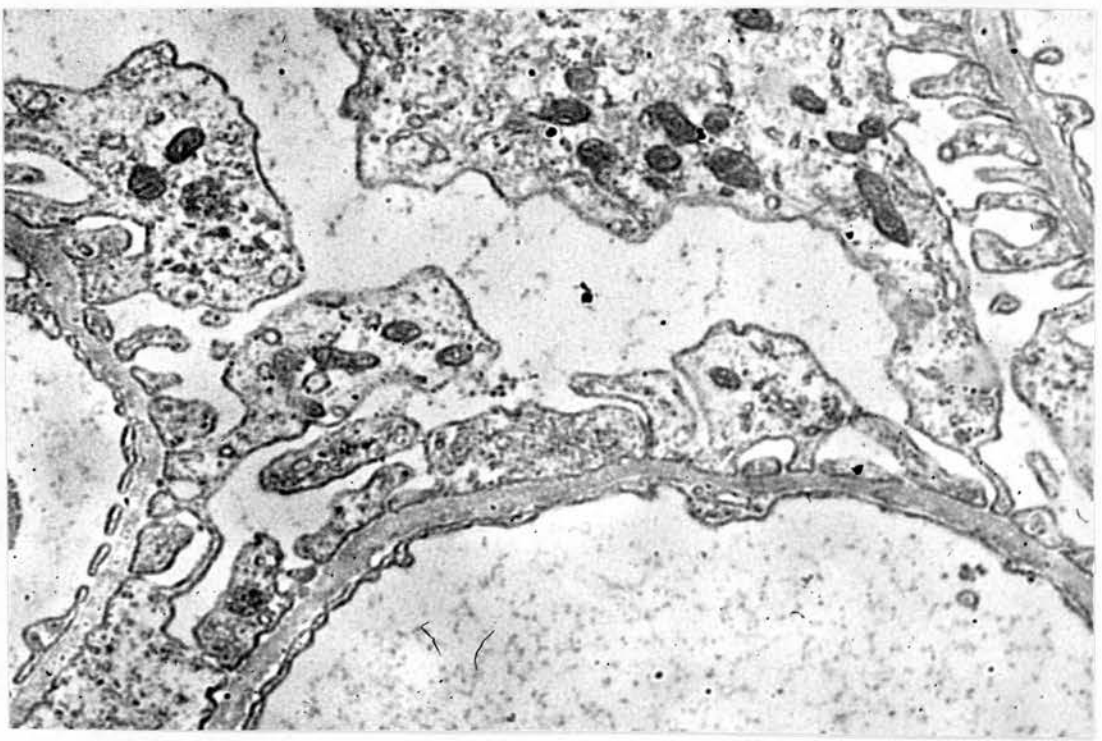


Fig. 16. Capillary wall from a normal albino rat's glomerulus.
x 16,000

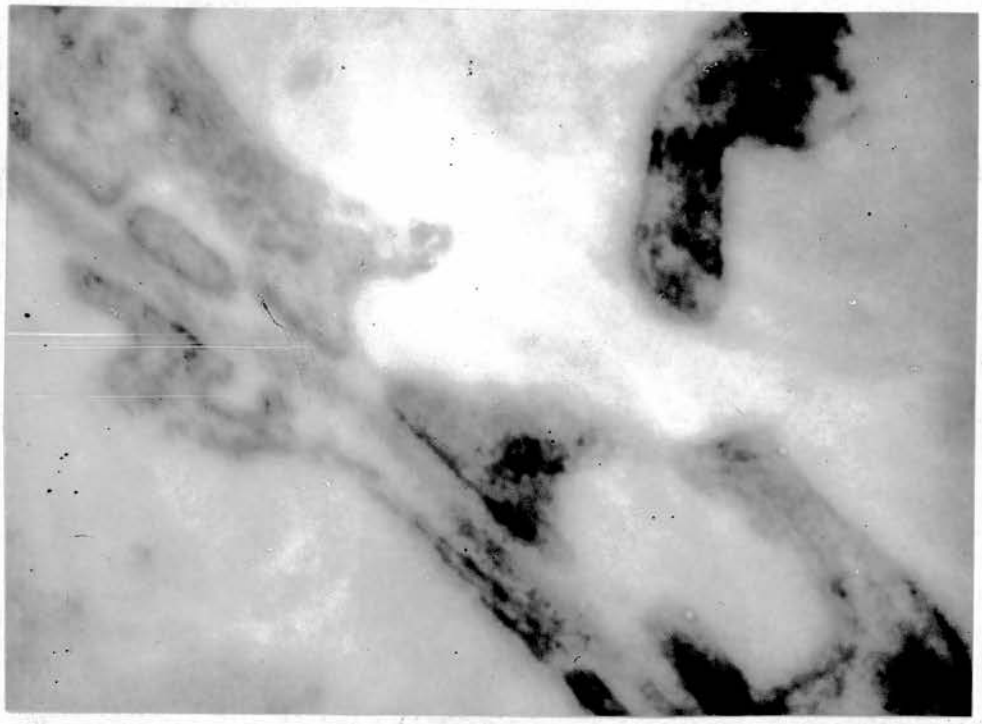


Fig. 17. Glomerular capillary wall from a normal adult rabbit's kidney. Note the filtration slit-membrane connecting the pedicels, and the clear areas on each side of the basement membrane in this imperfect preparation. x 90,000

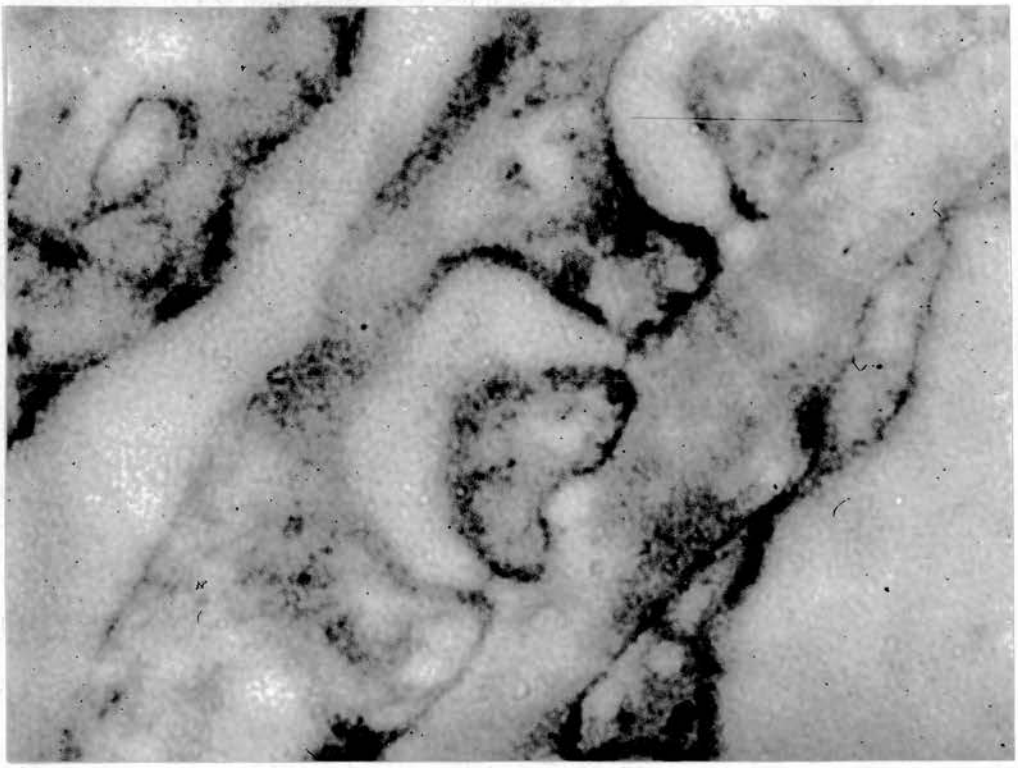


Fig. 18. Glomerular capillary wall from a normal adult albino rat's kidney. Note the filtration slit-membrane connecting the pedicels. The basement membrane fills all the space between the epithelial and endothelial cell membranes in this perfect preparation. The foot processes in the middle is in the process of pinocytosis. x 90,000

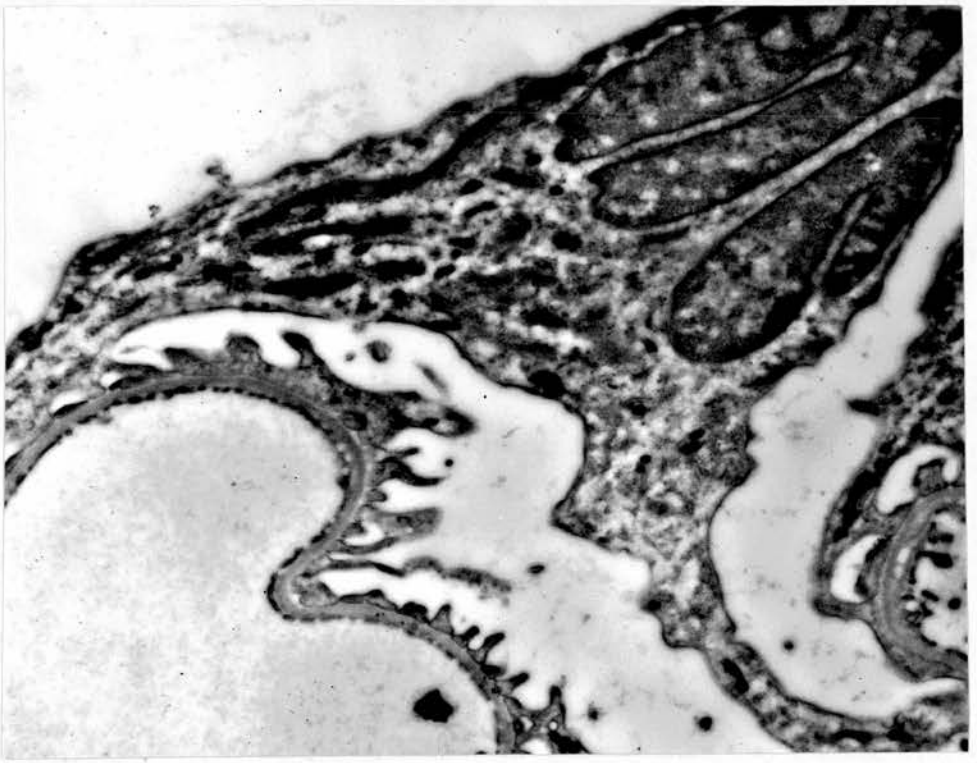


Fig. 19. Glomerular epithelial cell from an albino rat. Note the deeply indented nucleus. x 9,000

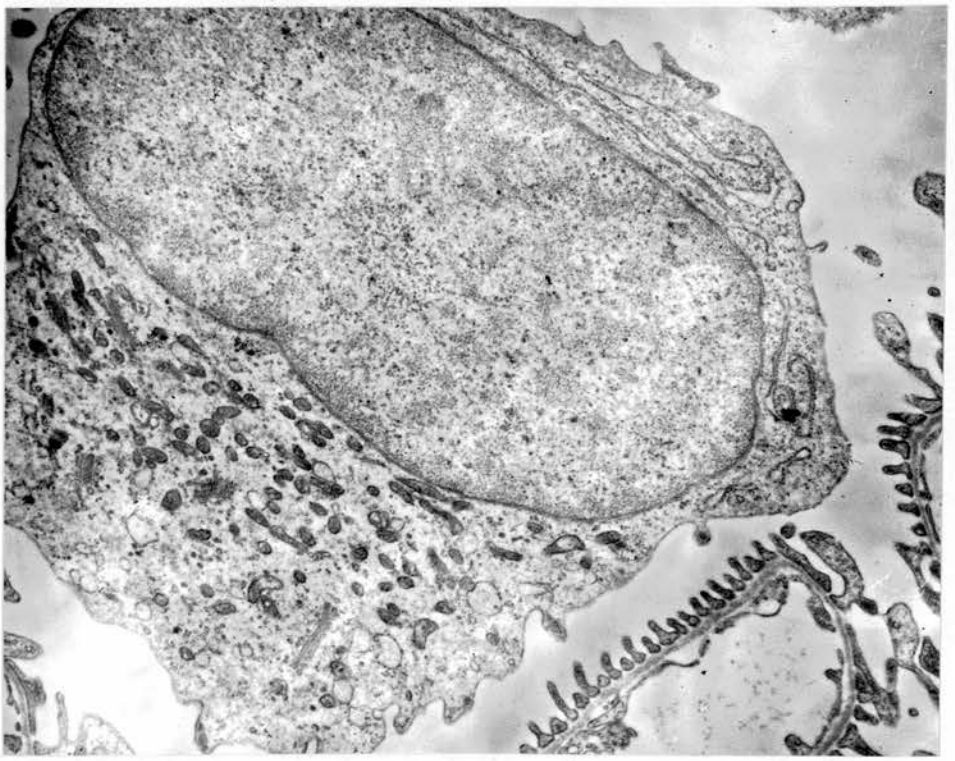


Fig. 20. Glomerular epithelial cell from a normal rabbit. Canaliculi of the endoplasmic reticulum are beautifully seen above the nucleus. Many pinocytotic vesicles and a pile of Golgi cisternae are seen below the nucleus. The mitochondria are quite small and relatively few. x 12,000

Fig. 21. Glomerular epithelial cell from a normal rabbit. The Golgi apparatus is very well seen in this electron micrograph. x 15,000.

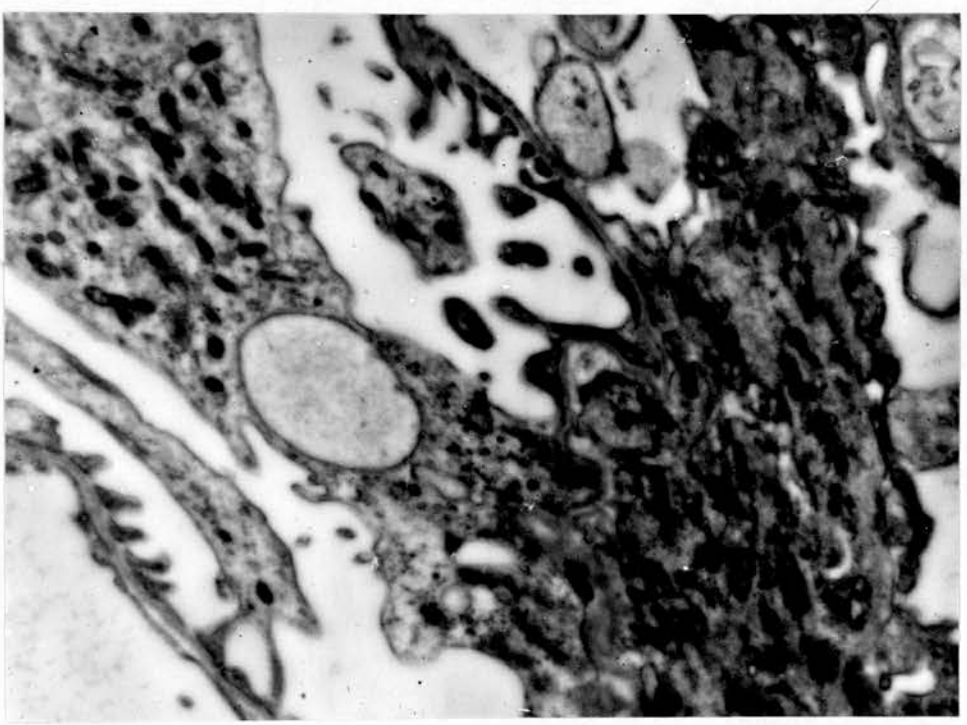


Fig. 22. Part of a glomerul ar epithelial cell from an adult rabbit. Many small vesicles are seen in the perikaryon and a large one is seen in the trabecula. x 15,000

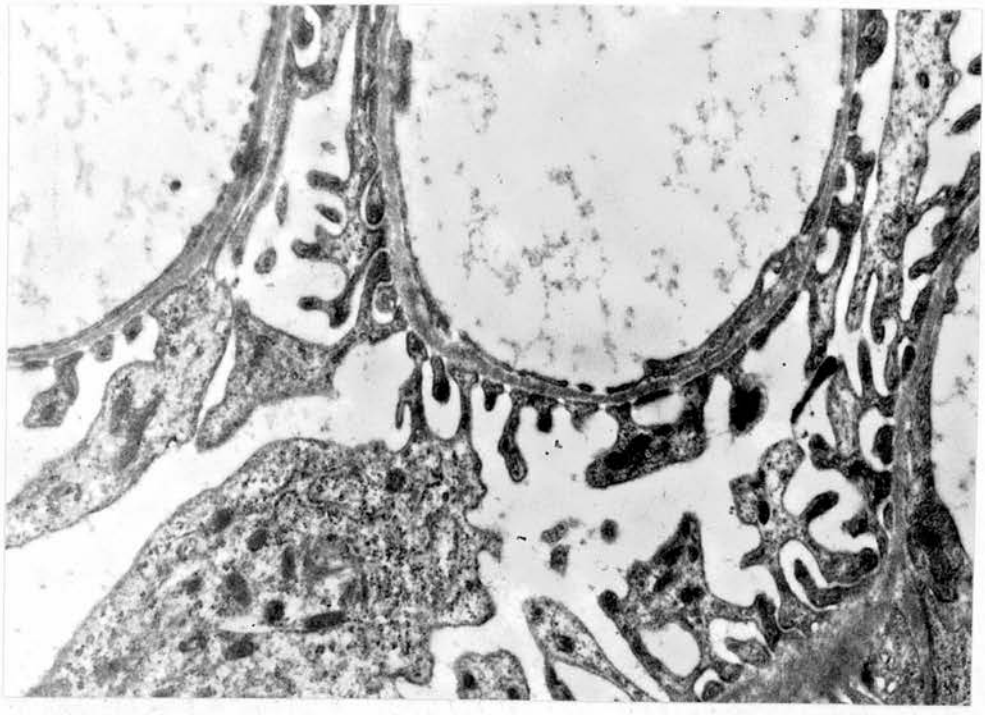


Fig. 23. Part of a glomerulus from a hooded rat. Two epithelial trabeculae are seen on the left and a third on the right, from all of which the foot processes arise. x 14,000

Fig. 24. A moderately low power electron micrograph to show the great length of the epithelial trabeculae. From a normal adult rabbit's glomerulus. x 6,000

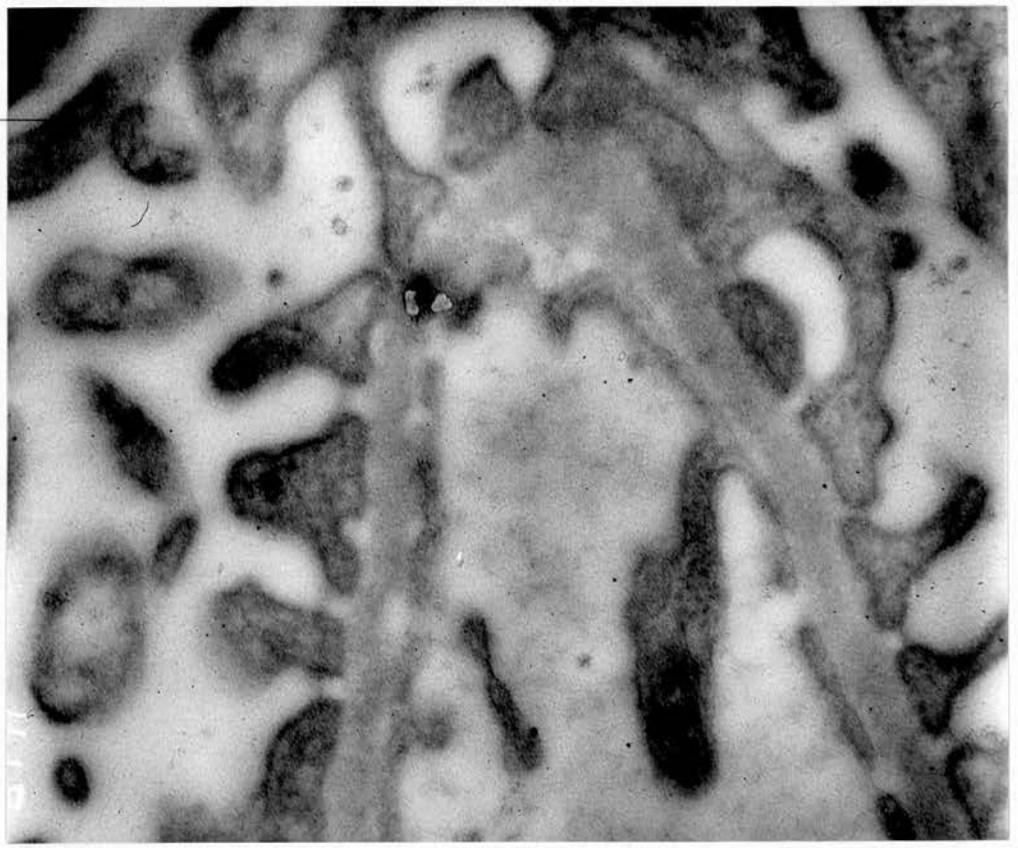


Fig. 23a. Capillary wall from a hooded rat's glomerulus, highly magnified, beautifully showing the filtration slit-membrane bridging the narrowest point of the gap between foot processes. x 75,000

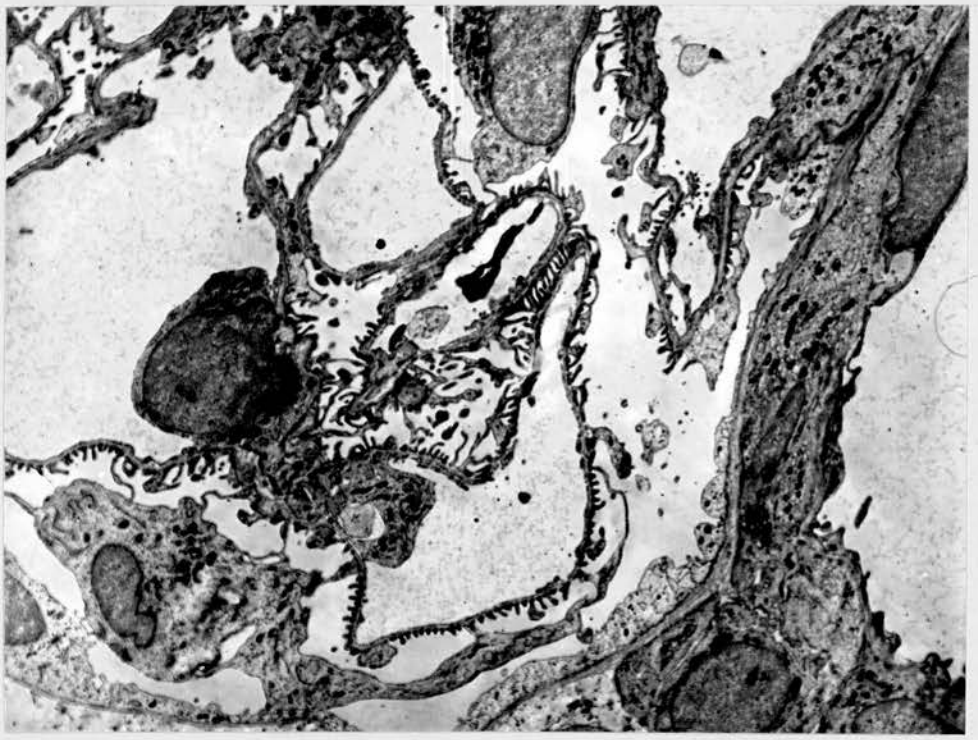


Fig. 25. A low power electron micrograph of a normal rabbit glomerulus. x 2,500

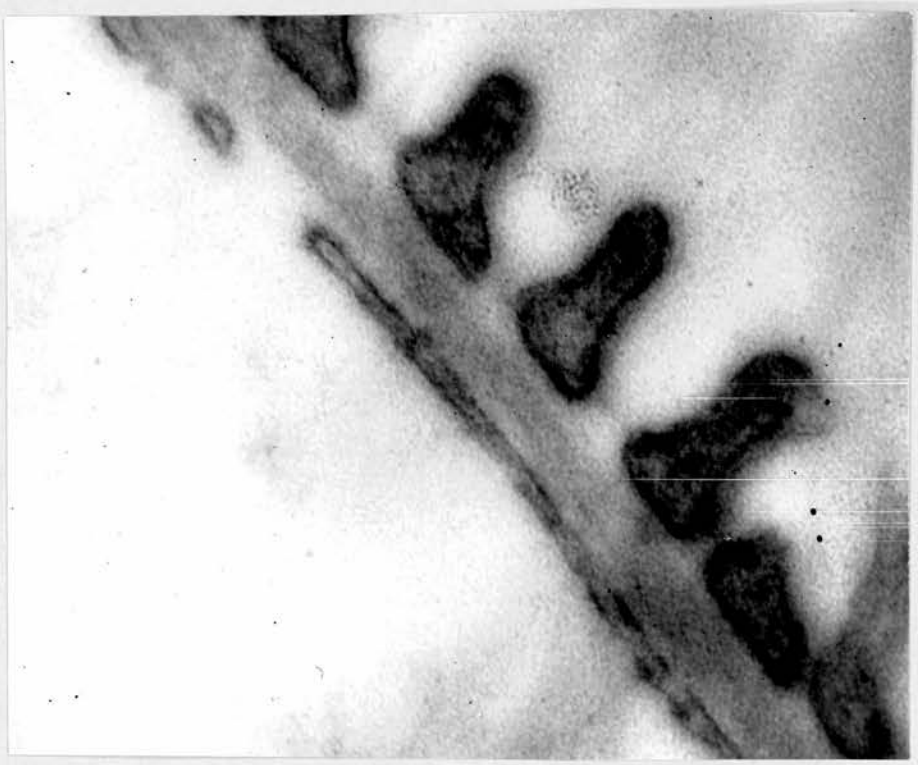


Fig. 26. Glomerular capillary wall of an adult rabbit. It consists of a basement membrane, lined by an attenuated, fenestrated endothelium and covered by foot processes. Note the broad expansion of the foot process in contact with the basement membrane. x 120,000

Fig. 27. Glomerular capillary wall of an albino rat. An invagination of the cell membrane of the foot process in the middle apparently represents a process of pinocytosis. x 90,000

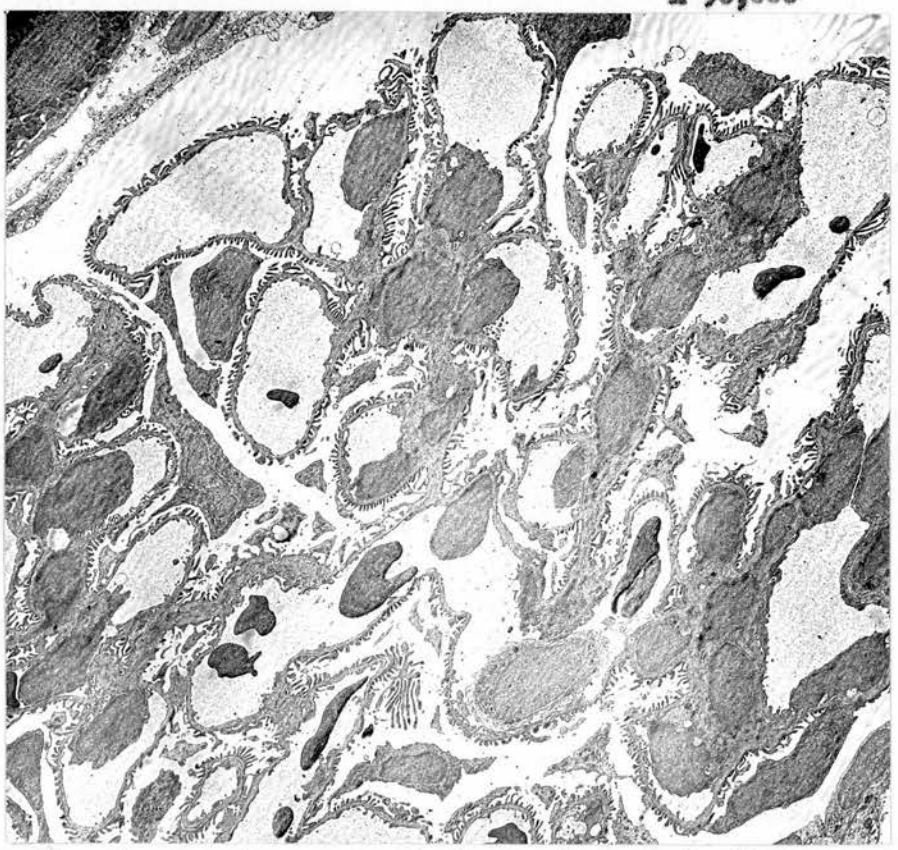


Fig. 26. A very low power electron micrograph of a glomerulus from a hooded rat. Note that the nuclei of the endothelial cells are mostly located in the axial region. x 1000.

Fig. 29. The axial region of a glomerulus from an albino rat showing piling up of endothelial nuclei. x 15,000

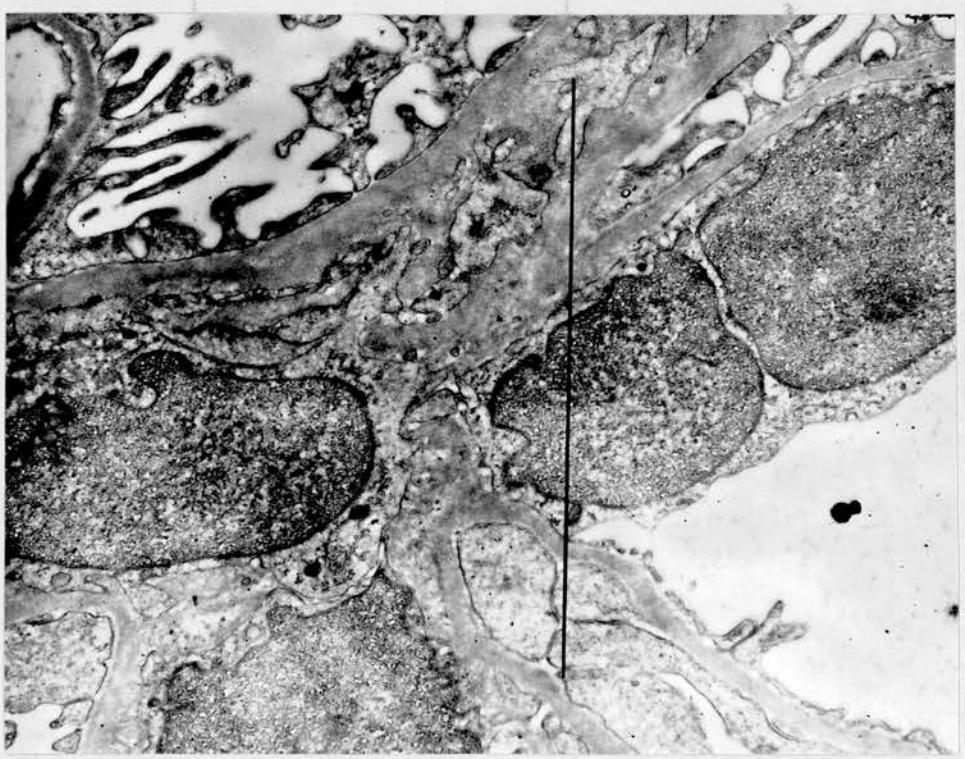


Fig. 30. The axial region of a glomerulus from an albino rat. The basement membrane is irregular and provided with spurs. The two cells on the left confirm what has been termed "intercapillary" cell. However, a section in the position indicated by the dark line and at right angles to the present plane of sectioning will cause the cut cell, which is definitely endothelial, in this plane, to appear as an "intercapillary" cell. x 12,000

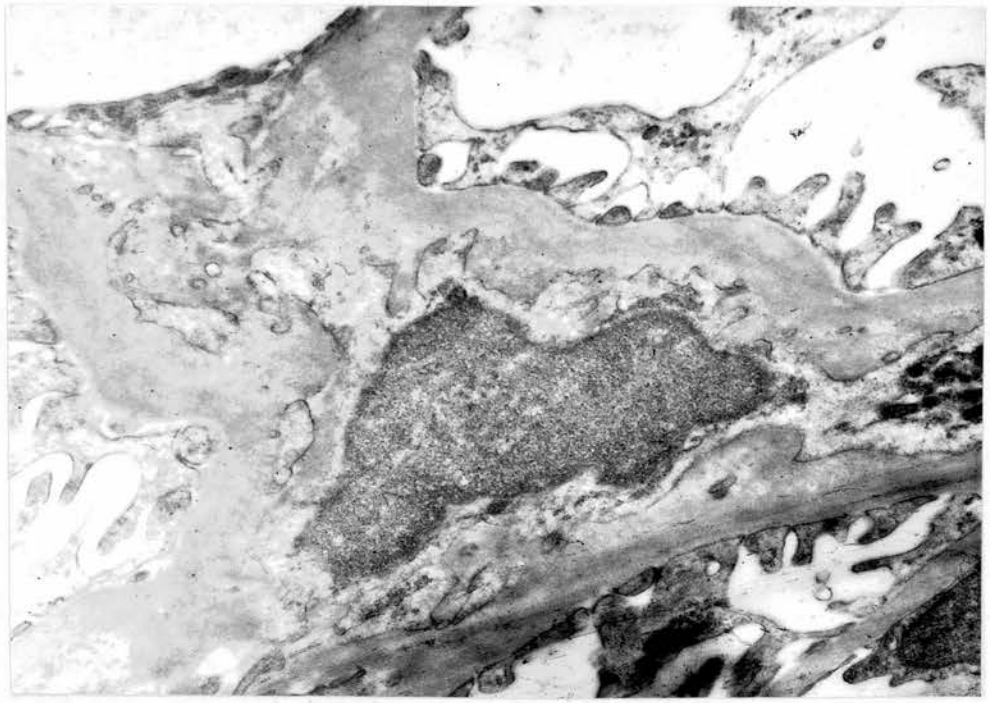


Fig. 31. The axial region of a glomerulus from an albino rat. A cell with a nucleus is seen in the centre, separated from the capillary lumen (upper left) by a layer of attenuated endothelial cytoplasm, and from Bowman's space (upper right, left and below) by basement membranes on which pedicels rest. x 15,000

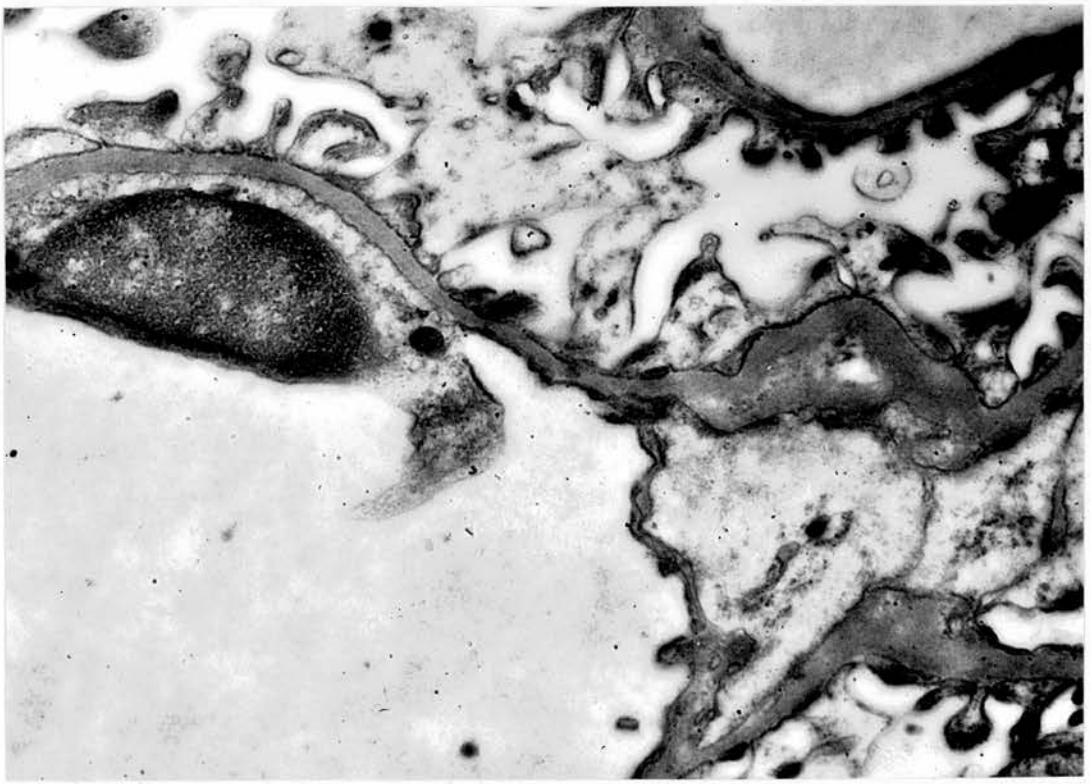


Fig. 32. Capillary from an albino rat's glomerulus. The mass of cytoplasm on the right side is separated from the lumen by a basement membrane, lined by an attenuated, fenestrated endothelium, and can be considered, judging from this alone, to be "intercapillary" in position. x 12,000

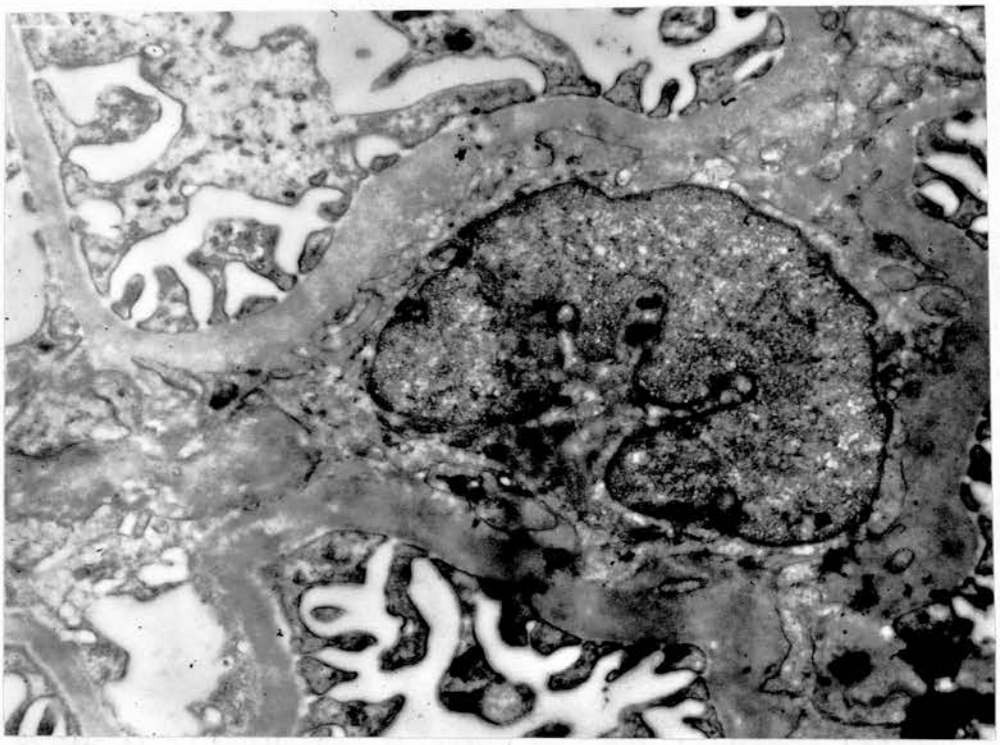


Fig. 33. A cell in the axial region of the glomerulus of an albino rat not contributing to the lining of a capillary lumen in this plane of section. x 15,000

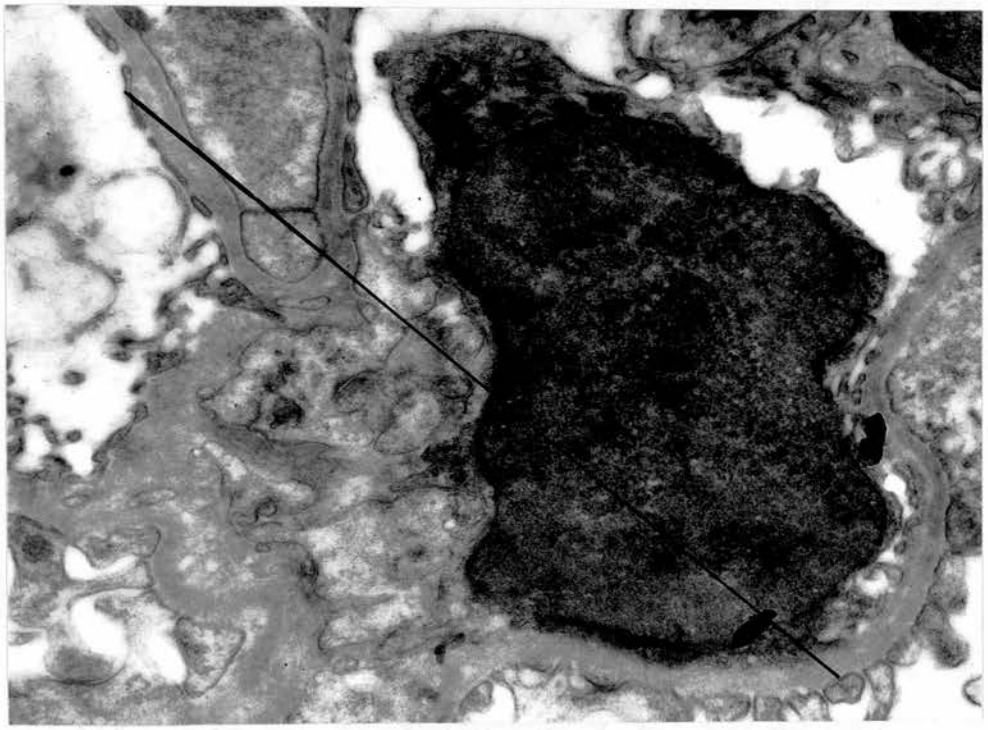


Fig. 34. Two glomerular capillaries from an albino rat. A section cutting the definitely endothelial cell in the corner of the capillary on the right in the line indicated and at right angles to the present plane of section will cause it to appear as if it were "intercapillary". x 24,000

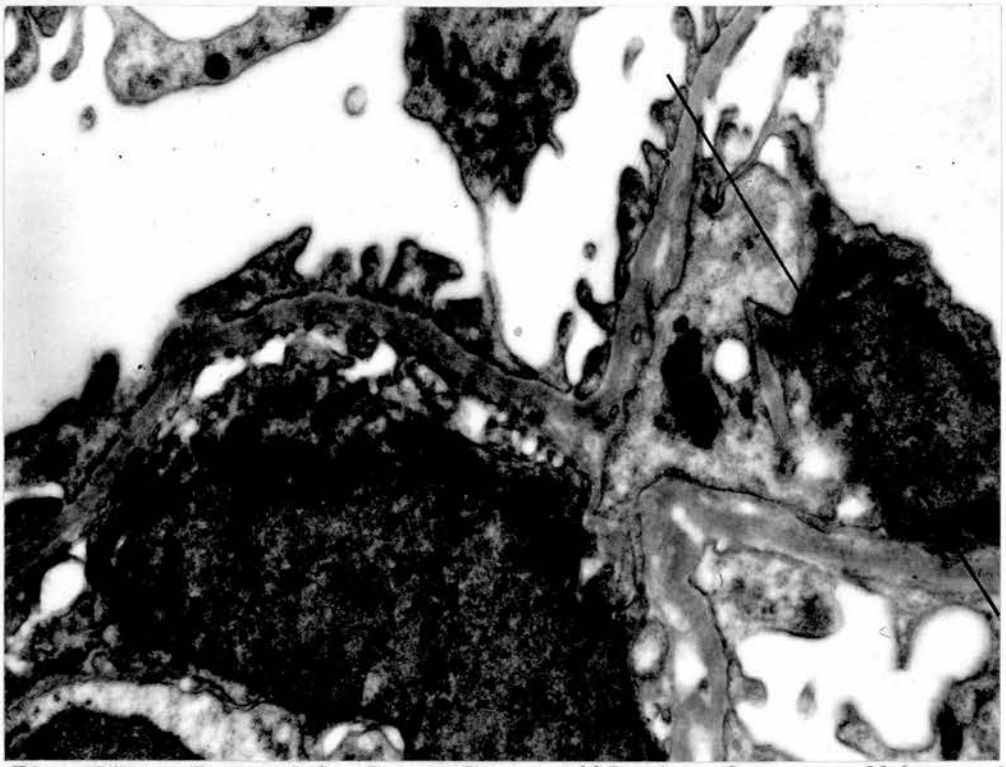


Fig. 35. Two axial glomerular capillaries from an albino rat. The definitely endothelial cell in the capillary on the right, if cut in the line indicated and at right angles to this plane of section will lose any apparent contact with the lumen and will appear "intercapillary". x 24,000

Fig. 36. Glomerular capillaries from an adult rabbit's kidney. A section in the endothelial cell, as indicated, at right angles to the plane of this electron micrograph will cause to appear "intercapillary", away from any lumen. x 6,000

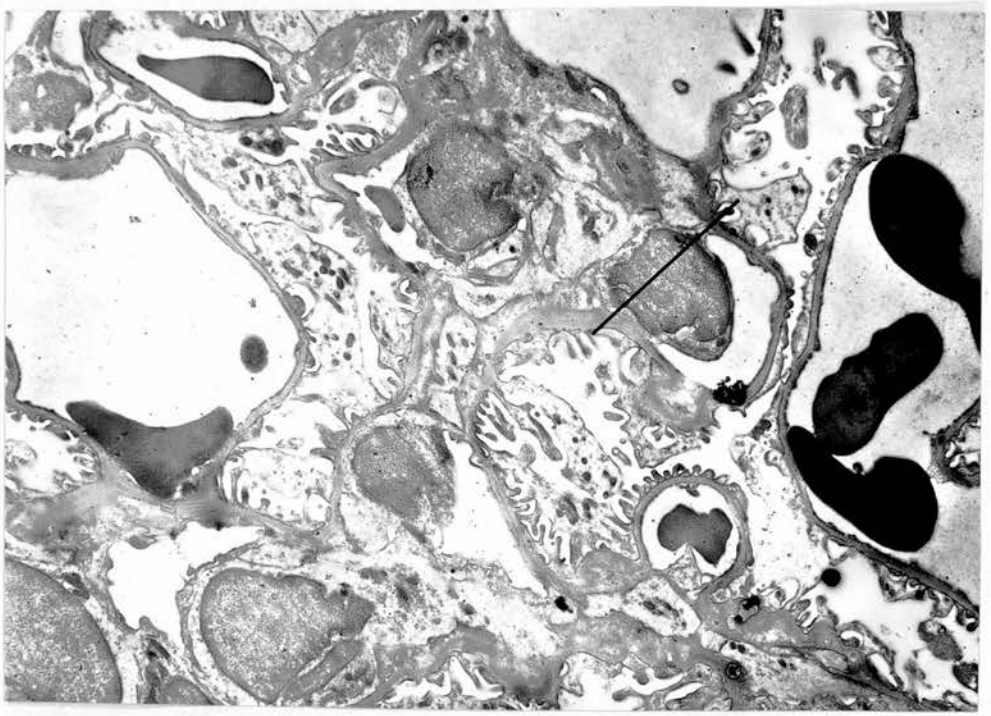


Fig. 37. Glomerular capillaries from an albino rat's kidney. A section in any endothelial cell at the corner of a capillary, at right angles to the present plane, might cause loss of direct contact with the lumen and the cells would then be regarded as "intercapillary". x 4,000

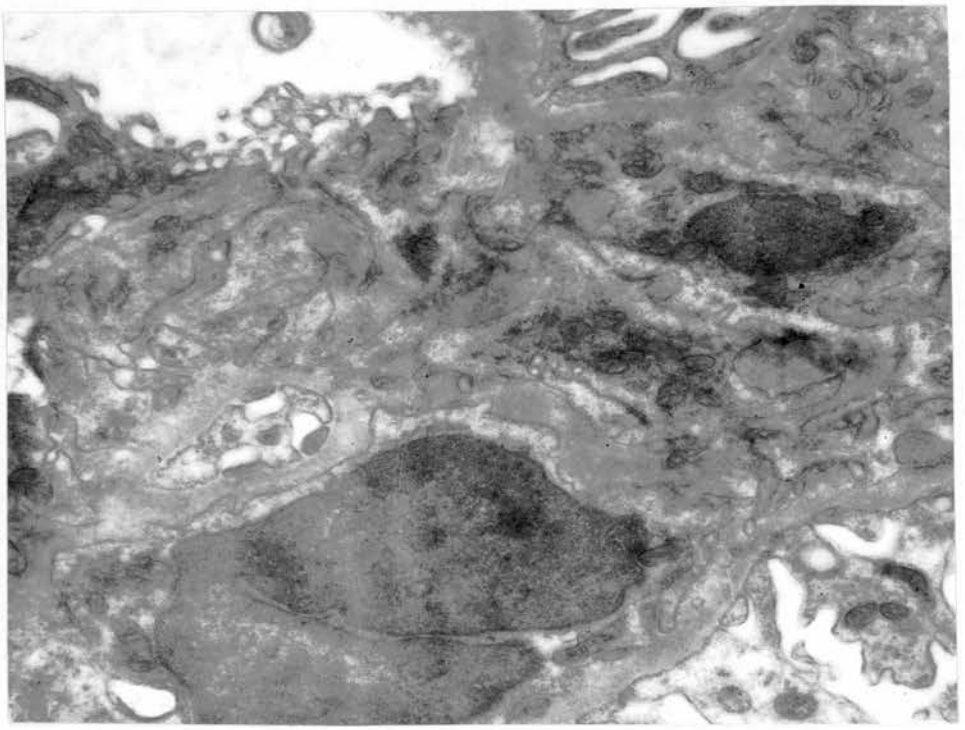


Fig. 38. In what is apparently an intercapillary mass, definite epithelial foot processes are seen. From an albino rat's glomerulus. x 19,500

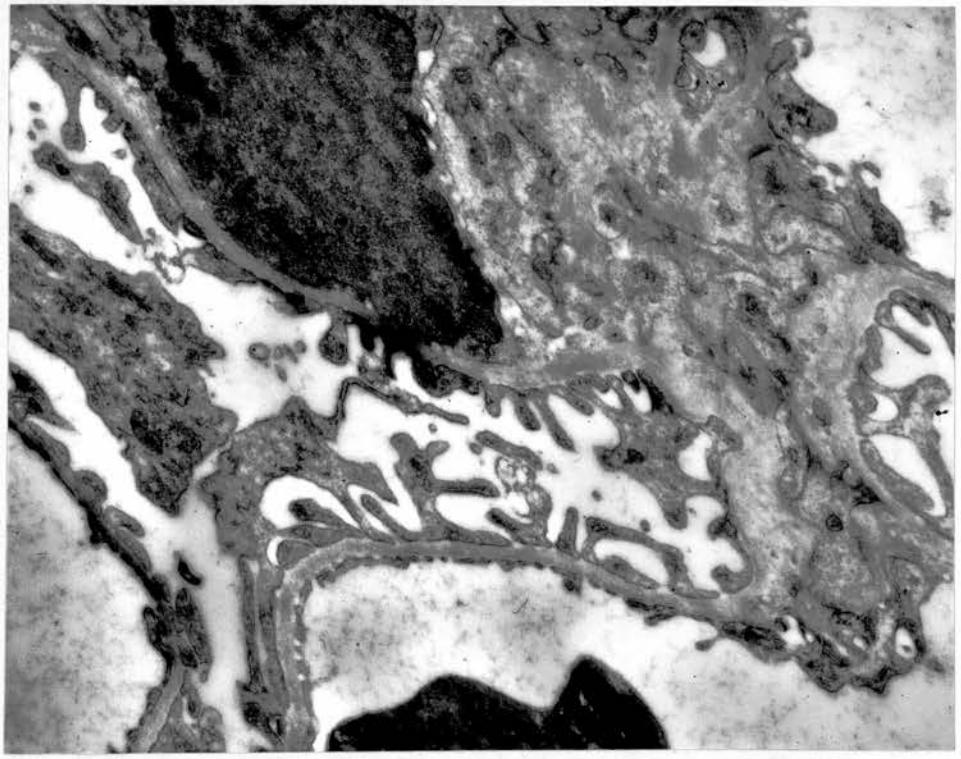


Fig. 39. Definite epithelial pedicels are seen amidst the so called "intercapillary" tissue. From an albino rat's glomerulus. x 15,000

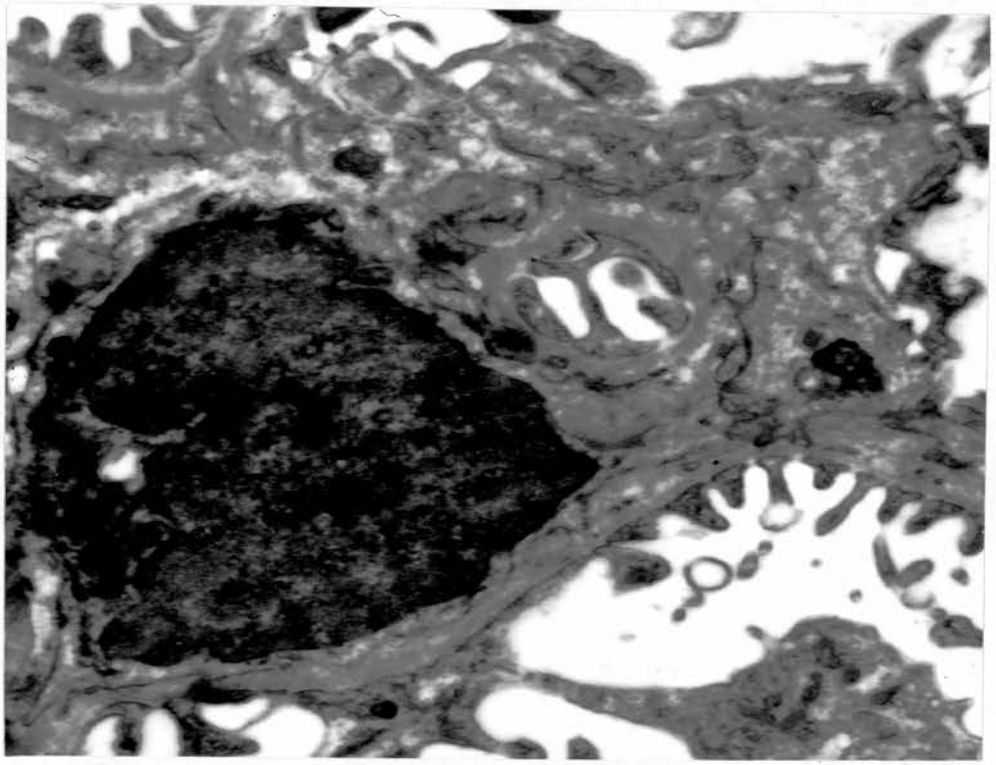


Fig. 40. Epithelial pedicels are seen in the centre of this "intercapillary" mass, revealing that the epithelium constitutes at least a part of it. From an albino rat's glomerulus. x 24,000

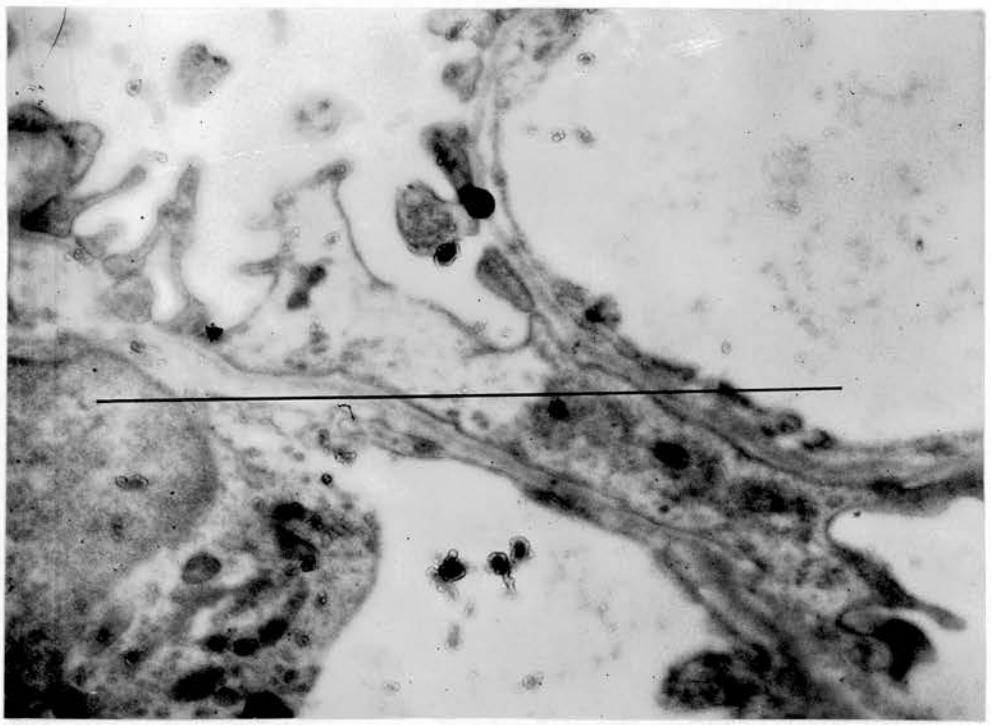


Fig. 41. Two glomerular capillaries from a normal rabbit's kidney. An epithelial trabecula is seen peeping between the two capillaries, in direct contact with both basement membranes. A section through the dark line and at right angles to the present plane would result in this trabecula as an "intercapillary" mass of cytoplasm, and its correct nature beyond recognition. x 24,000

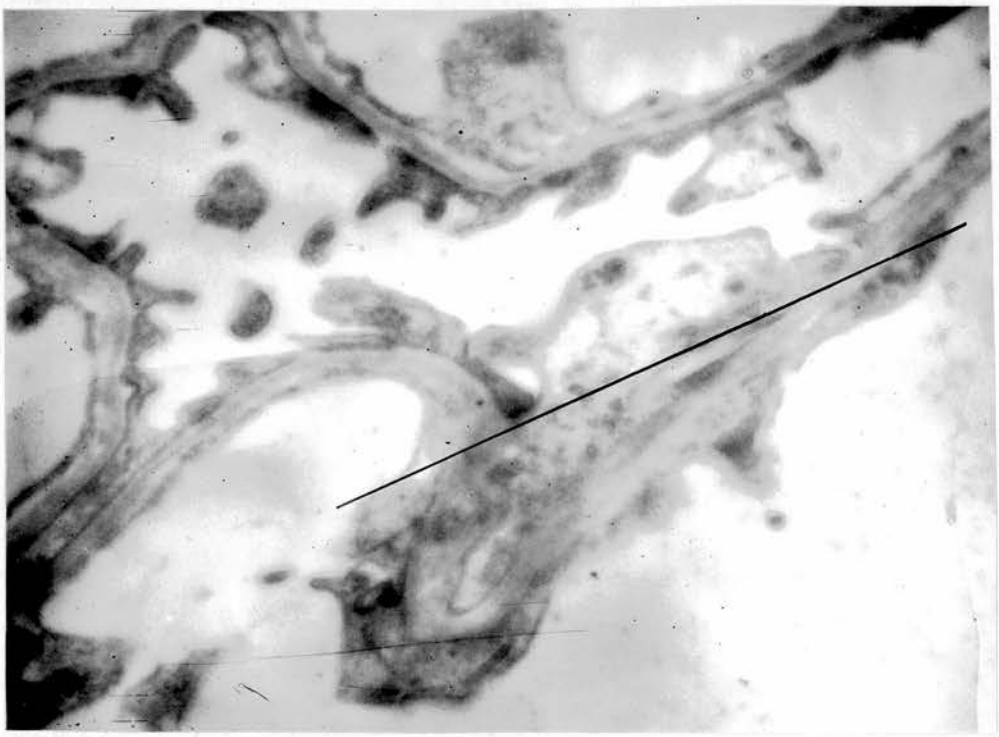


Fig. 42. Two adjacent capillaries from a rabbit's glomerulus. Two epithelial trabeculae can be seen peeping deeply between adjacent basement membranes. A section as indicated, and at right angles to the plane of this electron micrograph will make it impossible to identify theepithelial nature of the trabecula and the mass of cytoplasm will appear "intercapillary". x 24,000

Fig. 43. The definitely epithelial cells from this albino rat's glomerulus, will appear "intercapillary" if cut as indicated and at right angles to the present plane. x 9,000

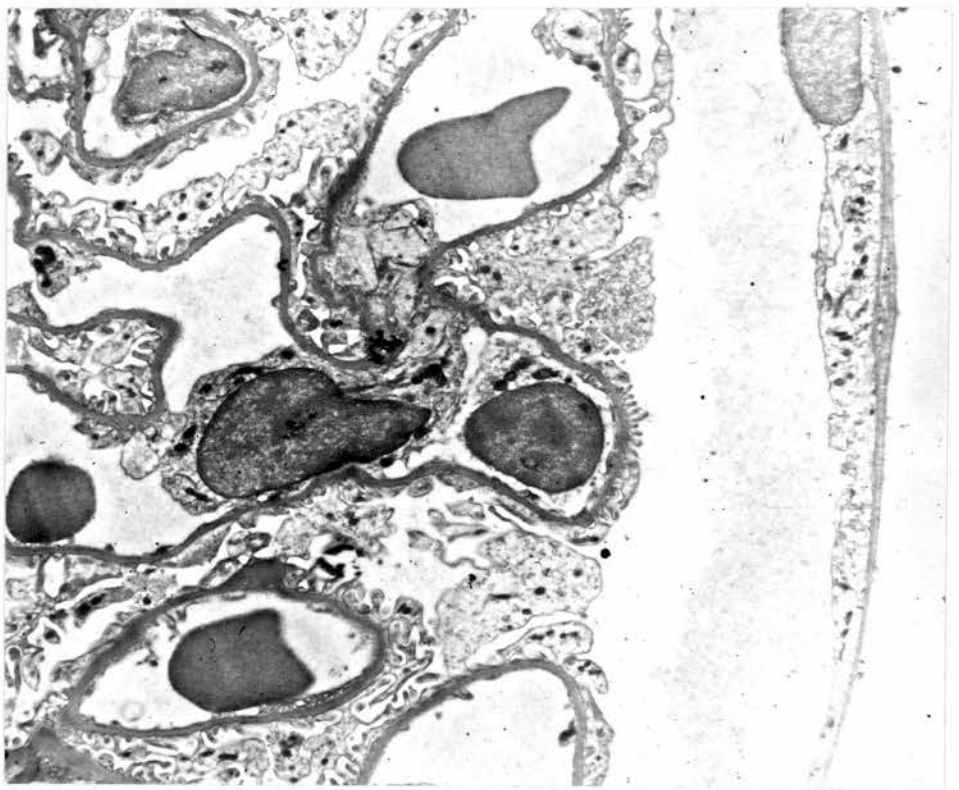


Fig. 44. Part of a glomerulus from a normal albino rat's kidney. Bowman's capsule is seen consisting of a fibrillar basement membrane lined by a simple, flat epithelial cell, with very few organelles. x 2,500

Fig. 45. Glomerular arteriole from a normal adult rabbit's kidney. x 6,000

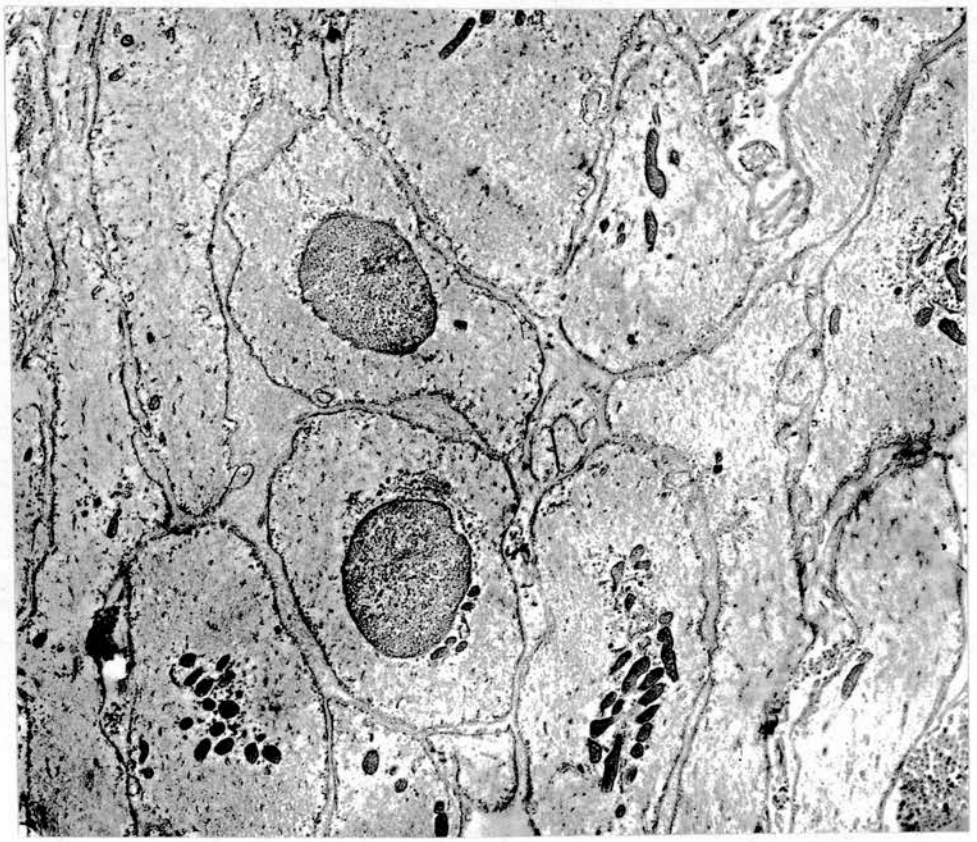


Fig. 46. Juxtaglomerular cells from the kidney of a forcibly hydrated albino rat. x 6,000

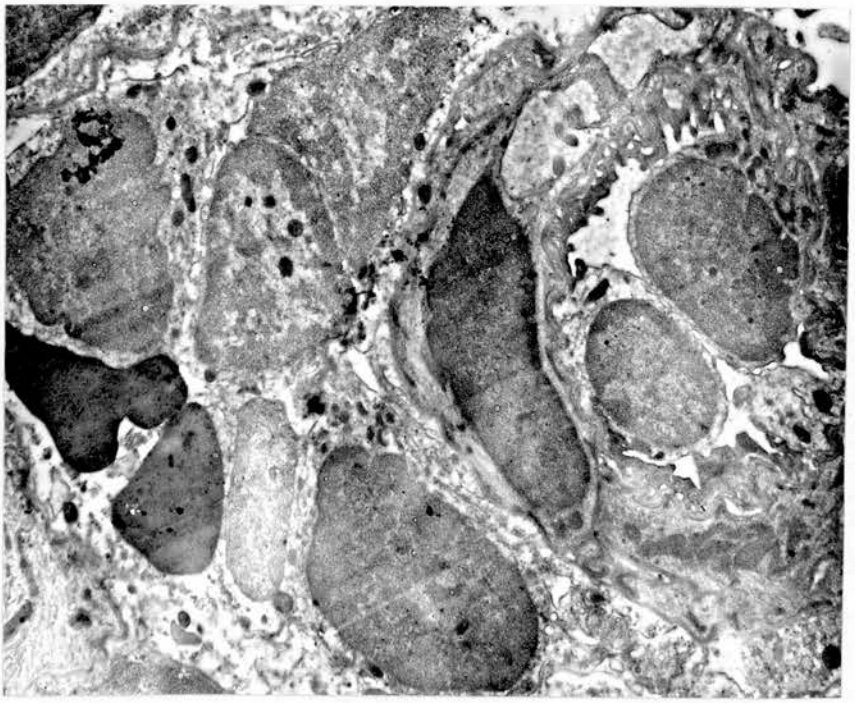


Fig. 47. Glomerular arteriole from a hooded rat. Note the juxtaglomerular cells with the dark granules in their cytoplasm. x 4,000

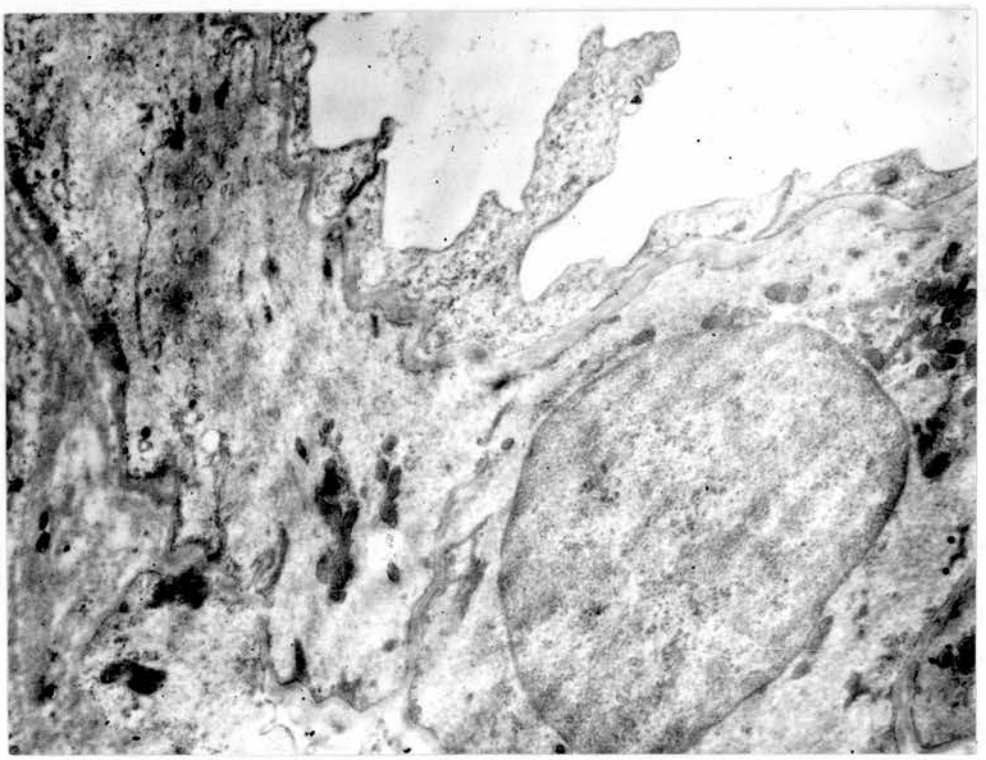


Fig. 48. Glomerular arteriole from a normal rabbit's kidney. An endothelial cell in the centre can be seen joining another cell on either side. The endothelial cytoplasm is full of pinocytic vacuoles. x 15,000

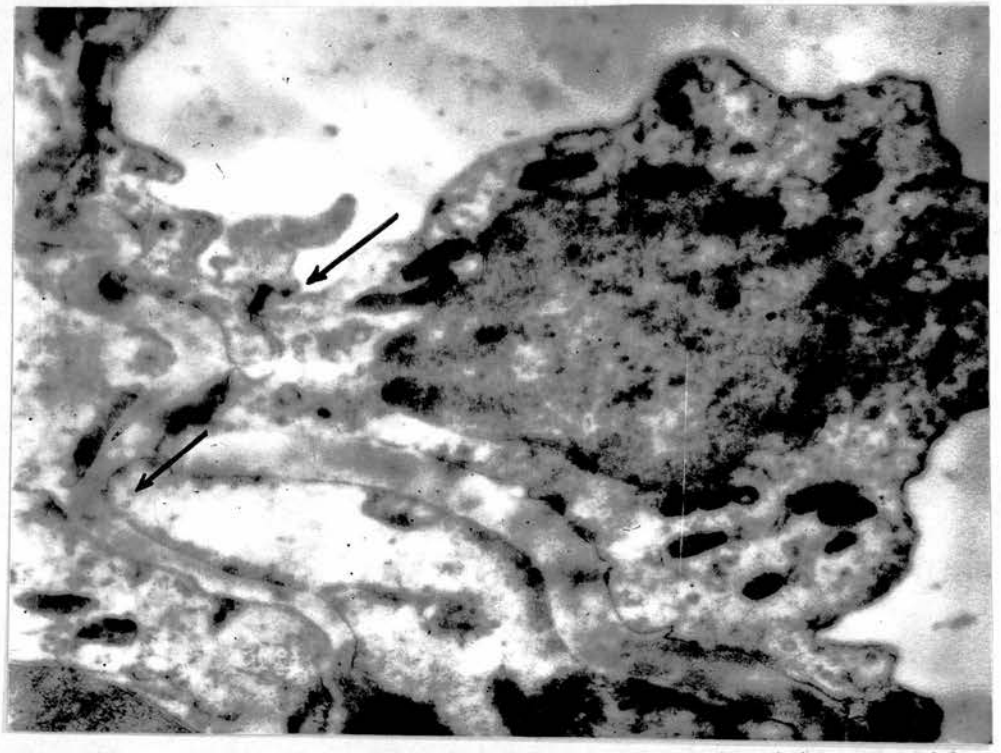


Fig. 49. Glomerular arteriole from a normal adult rabbit's kidney. An endothelial attachment belt is seen on the left side (arrow). Note the gap in the internal elastic lamina through which the endothelial cell and the smooth muscle cell are in contact (arrow). x 30,000

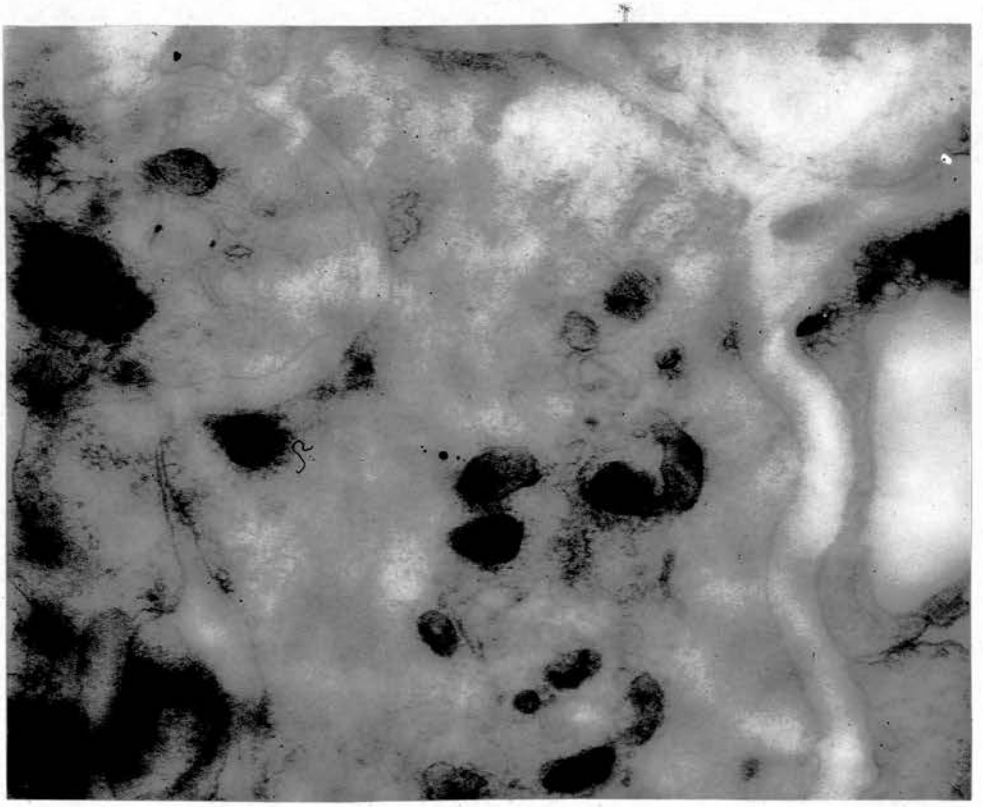


Fig. 50. Glomerular arteriole from a normal adult rabbit's kidney. The endothelium is seen on the right side (note the attachment bit) and the pinocytotic vesicles). Note that each smooth muscle cell is surrounded by an extension from the internal elastic lamina.
x 45,000

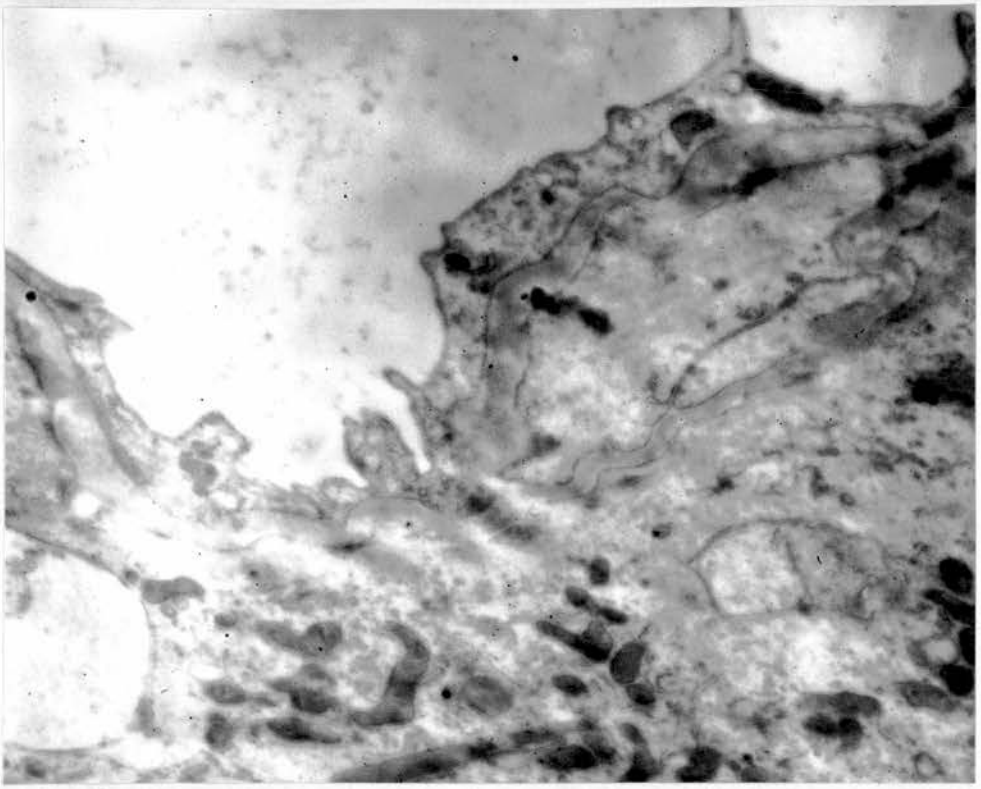


Fig. 51. Glomarular arteriole from a normal adult rabbit's kidney. The endothelial cytoplasm contains many pinocytic vesicles. A gap in the internal elastic lamina is apparent in the upper right corner. x 24,000

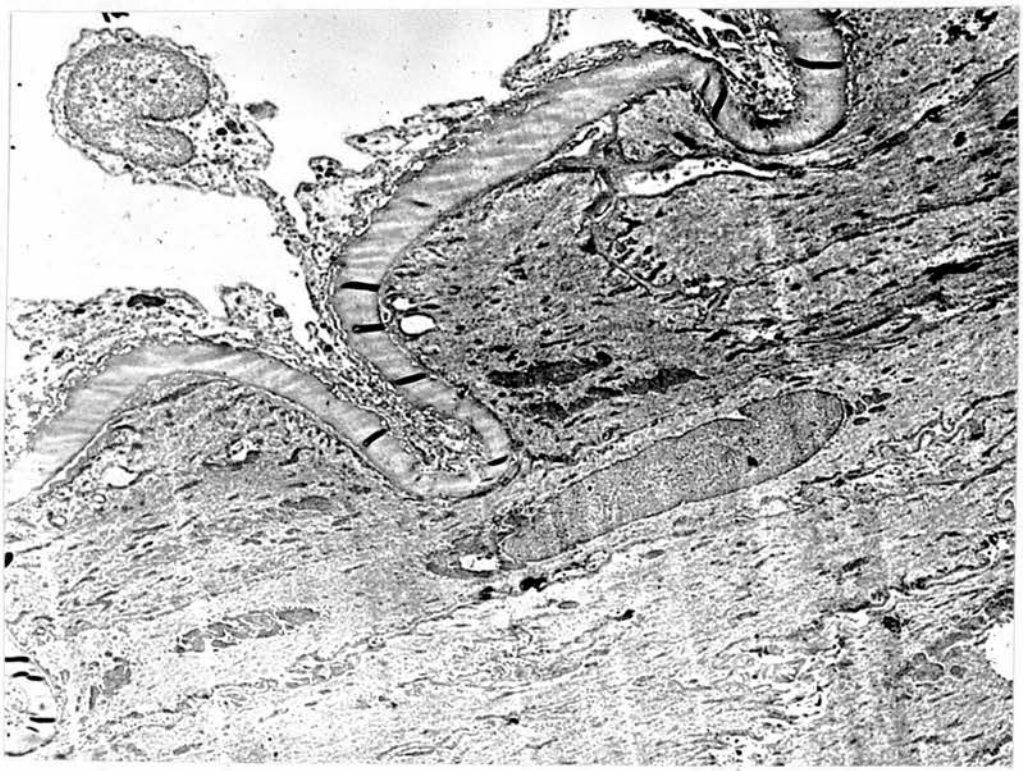


Fig. 52. Arcuate renal artery from an albino rat. The lumen is seen on the top left. An internal elastic lamina separates an endothelial cell on the luminal side from the smooth muscle cells. x 4,000

Fig. 53. Arcuate renal artery from an albino rat. Note the gap in the internal elastic lamina through which a tongue of the endothelial cell gets into direct contact with the muscle cell. x 9,000

Fig. 55. Juxtaglomerular cells from a forcibly hydrated albino rat. Note the pile of Golgi cisternae and the few mitochondria at the pole of the nucleus. x 15,000

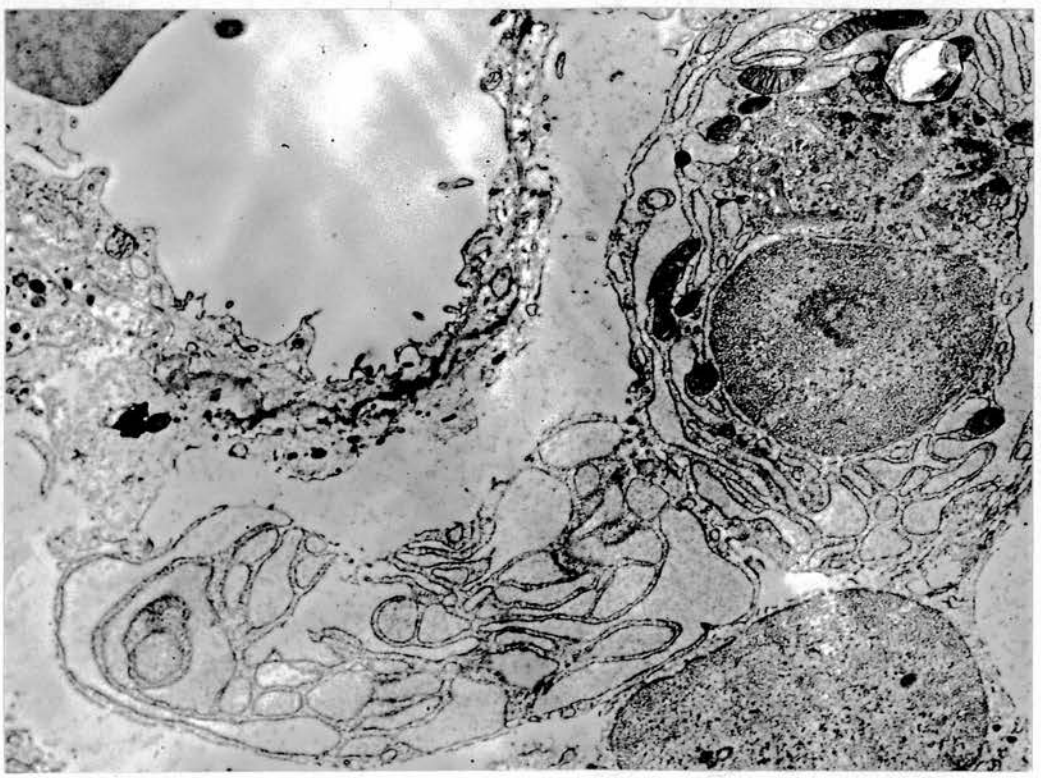


Fig. 56. Lacework cell from an albino rat. x 6,000

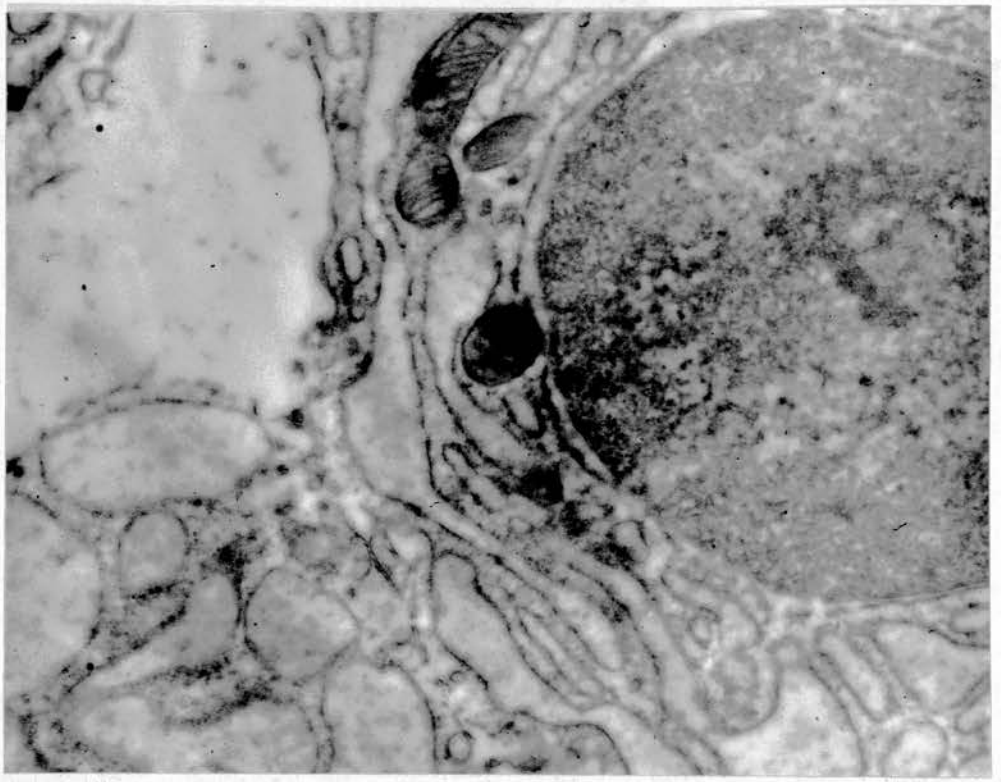


Fig. 57. Part of a lacework cell from the juxtaglomerular apparatus of an albino rat. x 24,000

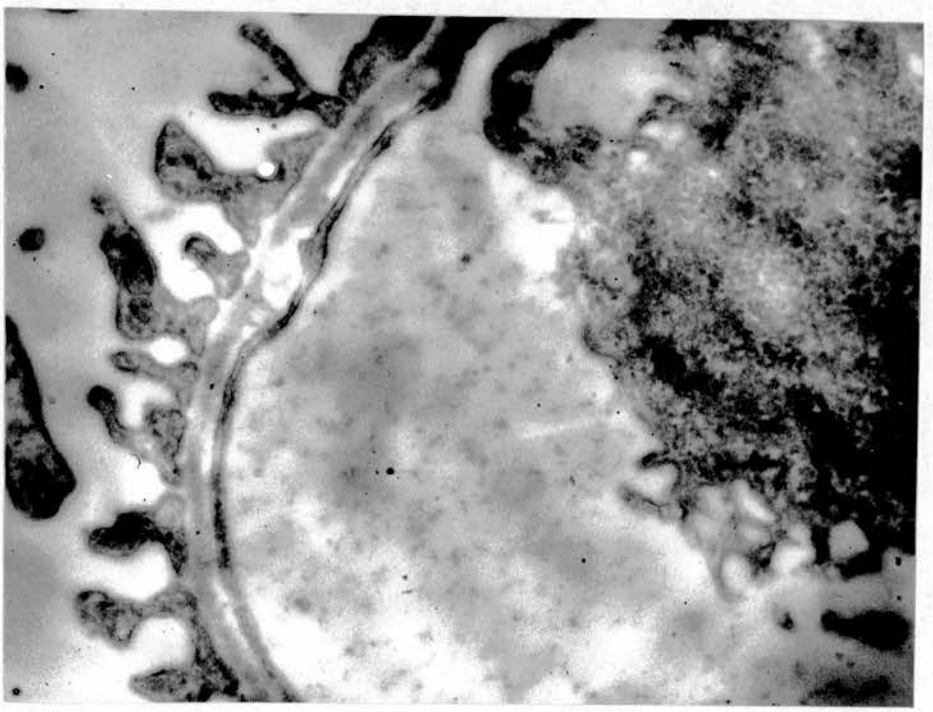
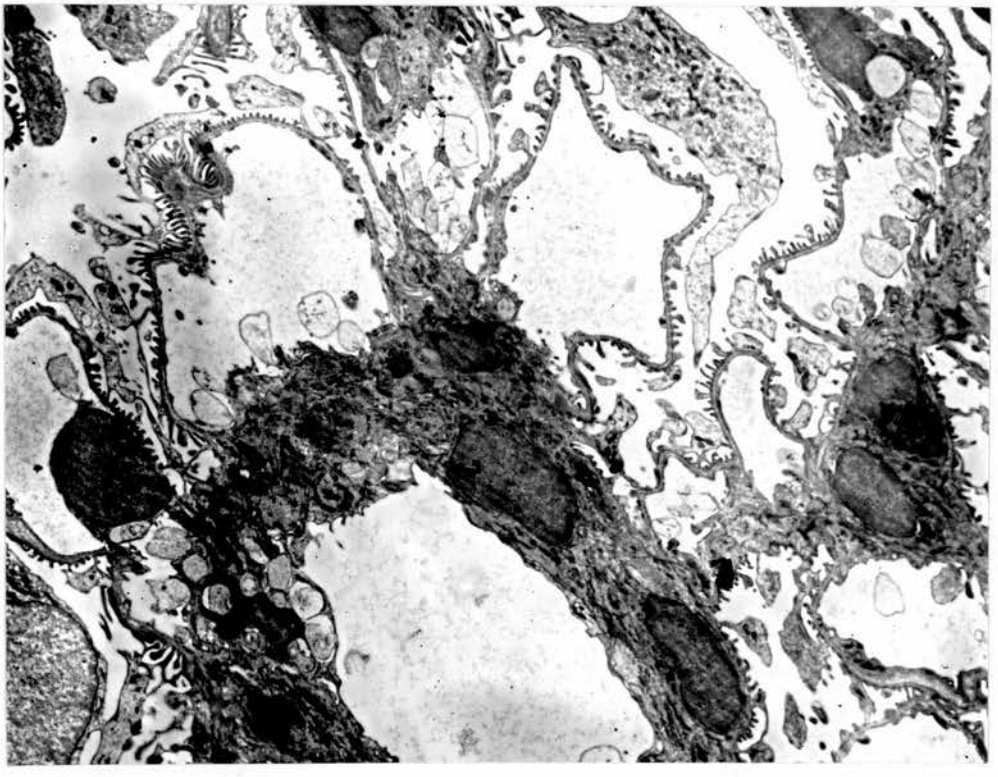


Fig. 58. Capillary wall from a hooded rat's glomerulus. Note the absence of endothelial fenestrae. x 30,000

Fig. 59. Axial endothelial cells from a normal rabbit's glomerulus containing numerous vacuoles filled with a granular material. In the lumina of many capillaries similar vesicles containing the same granular material is seen. x 4,000



DISCUSSION.

Electron microscopic studies have shown that the cell, especially the cytoplasm,has a much more complicated minute structure than had previously been supposed. The first surprising discovery was that the further one progresses in the sub-light-microscopic field the more the multiplicity of the basic cytomorphological phenomena decreases rather than increases. The minute cytoplasmic structure of the mammalian cells, for example, can be reduced to a limited number of structural elements which occur in all cells of whatever tissues. The morphological differences between one cell and another, which must obviously be somehow related to differences in function, lie not so much in the nature of the cellular structures as in their number and arrangement.

The Glomerular Arteriole.

All electron microscopists who have studied the glomerulus did not describe the glomerular arterioles.

The minute structure of the glomerular arterioles was found identical with that described for mammalian heart arterioles by Moore and Ruska in 1957 (98). The occurrence of vesicles in endothelial cells of the arteriole indicates that a process of pinocytosis is performed by these cells. The internal elastic lamina seems to be a strong barrier to diffusion as well as to ultrafiltration, apparently much more so than the basement membrane of the glomerular capillaries. In order to secure a nutrient supply to the smooth muscle cells, in absence of vasa vasora in these small arteries, fenestration of the internal elastic lamina and direct contact between the

endothelial cells and the smooth muscle cells through these windows exist and should seem to solve the problem. Therefore, the behaviour of the endothelial cells is not only one of pinocytosis (cell drinking) in which fluid is incorporated by the cell for its own supply, but also one of cytopempsis (transmission by cell) whereby substances are transmitted through the cytoplasm in addition to being solely utilised by the cell (98).

The Juxtaglomerular Apparatus.

Though the individual components of the juxtaglomerular apparatus have been described many years ago, it remained in relative medical obscurity, however, mainly because its cells (with the exception of the macula densa) cannot be readily seen in the usual haematoxylin and eosin histological preparations.

The modified muscle cells in the wall of the afferent arteriole were first described by Ruyter in 1925 (127) in the mouse and by Oberling in 1927 (104) in man and were extensively studied by Goormaghtigh (51) about twenty years ago, who suggested that they possess an endocrine function. It is now established that they are found in all mammals. In recent years the Hartrofts (65) have introduced a stain which clearly delineates the individual granules in these juxtaglomerular cells. Utilising this stain, it has been possible to determine that certain physiological states produce a hypergranularity of these cells, while other conditions are associated with a complete disappearance of the cytoplasmic granules. The macula densa was first described by Peter in 1907 (119) as a specialised part of the distal segment characterised simply by an accumulation of epithelial nuclei. Zimmermann, in 1933 (165) has the credit of giving the name "macula densa" to an elongated portion of the distal tubule composed

of narrow, tall epithelial cells with crowded nuclei. This segment he described as being found in the portion of the distal tubule where it touches the afferent arteriole, and reported that it was found in all mammals. Policard in 1950 (121) attributed to the cells of the macula densa an endocrine secretory function and described them as tall, epithelial cells with apical secretion granules.

The group of cells between the afferent arteriole, the macula densa and the glomerulus were considered by Goormaghtigh,(50)to whome we owe the predominant features of their structure, as "pseudo-meissnèrienne". These cells apparently constitute an important part of the "Polkissen" of Zimmermann, which he believes to extend into the glomerulus around the afferent arteriole. McManus (87) on the other hand believes that nothing intervenes between the macula densa and the granular cells in the wall of the afferent artériole, not even reticular fibres.

The whole juxtaglomerular complex was described by De Muylder (30) to be richly supplied with a network of nerves.

There are only two electron microscopic studies of the juxtaglomerular apparatus in the literature. The first, by Hartroft in 1956 (63) is a very short abstract with no pictures. The second, by Oberling and Hatt in 1960 (106) is a detailed study with which the results reported here completely agree except for a few minor modifications. In this study, Oberling and Hatt clearly described the complex lace-work cells and gave them the name "le lacis". The existence of a third type of cell in the juxtaglomerular complex has thus been clearly confirmed. Those authors have stated that these cells do not possess any of the characters of Schwann cells and none of the structural particulars which define nervous systems.

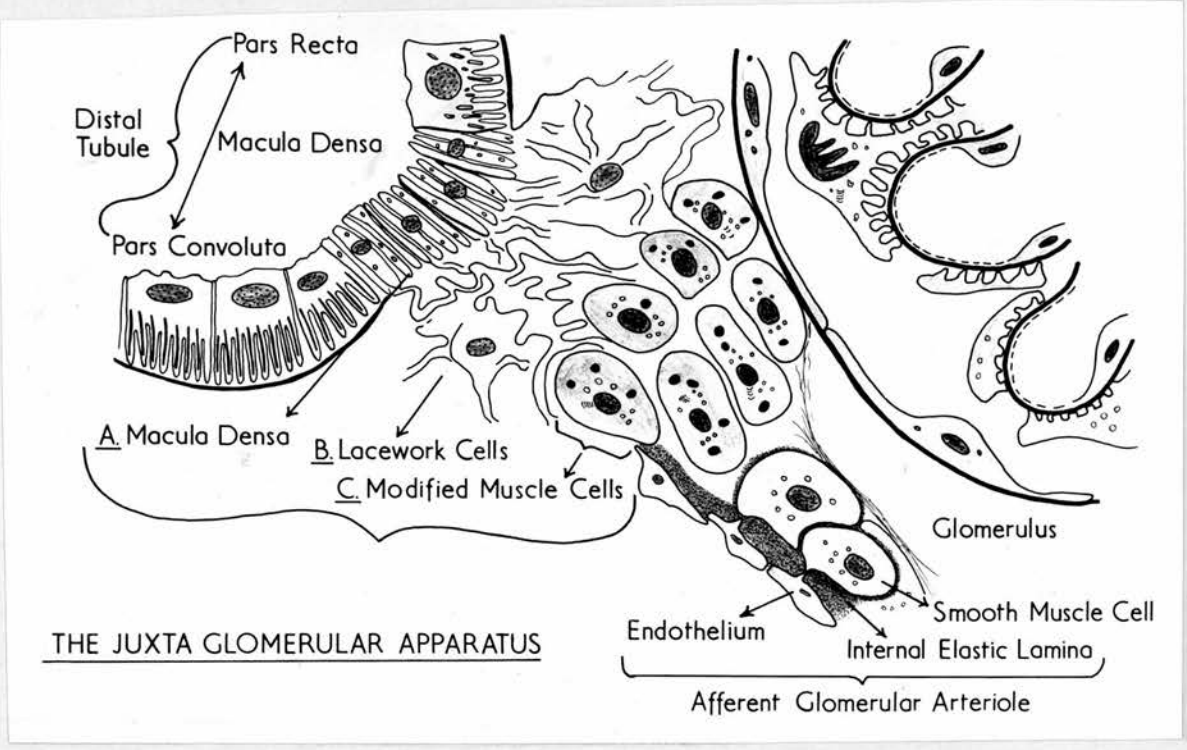

Pars Recta
Distal Tubule
Macula Densa
Pars Convoluta
A. Macula Densa
B. Lacework Cells
C. Modified Muscle Cells
Glomerulus
Smooth Muscle Cell
Endothelium
Internal Elastic Lamina
Afferent Glomerular Arteriole
THE JUXTA GLOMERULAR APPARATUS

Diagram 1.

Therefore, the original views of Goormaghtigh (50) and of De Muylder (30) have not been substantiated by electron microscopy. This also agrees with the experimental results of Tobian (154, 155) where isolated perfused kidneys were found to show the known variations in the degree of granulation of the juxtaglomerular cells in response to changes in the perfusion pressure.

The intricate structure of the juxtaglomerula apparatus, as revealed by electron microscopy, is very significant. (Diagram I). It clearly establishes a most clever morphological arrangement for an important physiological function.

The juxtaglomerular apparatus has been found to contain baro-receptors as well as chemoreceptors (154). Its structure suggests that it functions as an auto-regulation system for the chemical composition of the urine without nervous intermedium.

The location of the granulated cells in the wall of the afferent arteriole allows them to appreciate readily the changes in the stretch of the arteriolar wall. Many observations support the notion that they do act as stretch receptors. Degranulation of these cells was found to result whenever the pressure in the renal arterial system becomes elevated. Conversely, in situations where the pressure in the renal arterial system is reduced, either as a result of applying a clamp to the renal artery or from "curing" the hypertension, their granularity increases (154). Degranulation of these cells is probably related to their secretion of renin. Goormaghtigh (50) theorised twenty years ago, without much evidence, that these juxtaglomerular cells secreted renin. Recently, this hypothesis has been more or less proven (24, 64, 154). It is conceivable therefore, that these cells should be implicated in any phenomenon which seems

to depend upon the variations in the distension of the renal arterial system, reacting by secretion of a humoral pressor substance. Among such phenomena one could mention the pathological conditions of renal artery stenosis and malignant hypertension and the physiological phenomenon of circulatory "autoregulation" of the kidney.

Haddy and co-workers in 1958 (56) and Semple and de Wardener in 1959 (142), have demonstrated the constancy of the renal blood flow and of the glomerular filtration rate in the face of widely varying arterial pressures. This "autoregulation" of flow and filtration is active over the range of 75 - 200 mm. Hg (56). Variations of pressure in this range seem to affect the secretion of the juxtaglomerular cells (155), and consequently the tonus of the arteriolar muscle cells thus increasing or decreasing the resistance to flow and thereby would prevent a change in the filtering pressure in the glomerular capillaries as well as prevent a change in total renal blood flow.

The complex arrangement linking these granulated cells with the macula densa is also very remarkable. It allows small amounts of fluid to be sampled from the lumen of the distal tubule, after its chemical composition has been altered by the vital activity of the cells of the thick segment of the ascending limb of the loop of Henle, (the main or perhaps the only sodium pump responsible for the creation of a hyperosmotic papilla in association with the countercurrent multiplier system provided by the loop of Henle). Such small urine samples taken by the cells of the macula densa are then transferred by the canaliculi of the lace-work cells to thecanals in between the granular cells. Bathing of these cells individually bythe tubular fluid, directly handled to them, can well be conceived to affect their secretory function according to the chemical

composition of the bathing fluid. It has been shown that these granular cells respond to variations in the amount of mineralocorticoids in the body, as well as to varying amounts of sodium chloride in the diet (162) and it was suggested that the juxtraglomerular cells might be intimately related to the regulation of sodium excretion by the kidney (154). The ultrastructure of the juxtaglomerular apparatus conforms with the optimum device whereby the chemical composition of the tubular fluid can regulate the circulation in the glomerular capillaries and consequently the glomerular filtration rate without nervous factors playing a role; the macula densa being the sampling device, the ground substance canaliculi in the lace-work cells the transport system and the granular cells the glandular chemo-receptors.

It is interesting to report that degranulation of these juxta-glomerular cells was observed in a forcibly hydrated rat, 1½ hours after intragastric administration of 20 ml. of water. At this time the rat was maximally diluting its urine as evidenced by measurement of the urinary osmolality (vide infra). In this experiment two factors could be incriminated to have played a role on the juxtraglomerular cells; a sharp rise in the blood volume and consequently in the volume of blood in the renal arterial system, and a sharp fall in the sodium concentration of the tubular fluid bathing these cells.

If the juxtaglomerular apparatus did sense blood volume changes and also influenced sodium excretion, it would qualify as one of the long-sought after "blood volume and extracellular fluid volume regulating centres", for which there are many evidences pointing to its location in the renal arterial system (154).

The Glomerular Capillaries.

In the present study, confirmation of the well-known structural characteristics of the glomerulus has been obtained. In addition, some morphological features not previously reported have been observed, e.g. the manner of attachment of adjacent glomerular endothelial cells has been described in detail. The complex organisation of the epithelial slits reported in 1961 (44) has also been confirmed.

Since the glomerular filtrate is an almost completely protein-free watery solution, there must be an effective filter in the glomerular capillary wall capable of retaining substances of high molecular weight into the circulation. The fact that both the lining endothelium and the covering epithelium are discontinuous, and that the glomerular capillary basement membrane appears to be the only continuous layer between the blood and Bowman's space, it must be the filtration barrier.

The "slit-pore" hypothesis put forward by Hall (60) is most unlikely. Hall maintained that the pores between the foot processes represent the pores of the ultrafilter. If we apply the idea of a simple sieve, then the measurements involved are in themselves against Hall's hypothesis. Albumin molecules, the smallest protein molecules, are 150 Å long by 40 Å wide (153). Even if the pores between the pedicels were on average only 100 Å across as Hall maintains (60), they would still scarcely be small enough to act as a filter. All other investigators who studied the renal glomerulus by the electron microscope, including the studies reported in this thesis, agree that they are much wider (Table 3).

Table 3.

Author	Diameter of slit pore
Rhodin (127)	500 - 1000 Å
Siadat-Pour (143)	400 - 600 Å
Bargmann and co-workers (6)	350 - 400 Å
Yamada (164)	200 - 300 Å
Thoenes (153)	160 - 420 Å
Sabour (This study)	150 - 400 Å (average 250 Å)

Apart from this, Hall's hypothesis fails to take into account thefact that in pathological conditions characterised by proteinuria, the slit pores between the pedicels are often reduced in size or completely absent. The experimental demonstration by Farquhar and her co-workers (44) that ferritin molecules 100 Å in diameter and colloidal gold injected intravenously were effectively trapped mainly in the inner half of the basement membrane, is entirely against Hall's slit-pore hypothesis.

All these evidences point to the basement membrane as the main filter. Basement membranes are glycoproteins, consisting of a protein and muco-polysaccharide matrix within which fibrils are embedded. The latter are arranged parallel to the surface of the membrane and form a fine meshwork (102). These features have been confirmed by polarised light microscopic studies for the basement membrane of cerebral capillaries (102). On the basis of this structure Sitte (144) has put forward the theory that the meshes of the fibrillary network within the basement membrane represent the long sought-after filter pores responsible for the filtration. Thickening of the basement membrane would thus mean a widening of the network and

consequently an enlargement of the filter-pores. This hypothesis is particularly attractive because it helps to explain the paradoxical fact that in many pathological conditions thickening of the glomerular capillary basement membrane is associated with increased permeability to proteins.

However, no pores have been demonstrated in the basement membrane, though from Pappenheimer's calculation that the size of the effective filter pore is 30-45 Å, they should be well within the power of resolution of the electron microscope. Similarly, there are few conditions of proteinuria in which the glomerular basement membrane is not thickened; postural proteinuria and congenital nephrosis are two examples which I have personally seen. Moreover, in the experiments reported by Farquhar and her associates where a tracer was used (44), the ferritin molecules were capable of advancing through the basement membrane in the absence of visible pores of appropriate dimensions. The molecules did not follow preformed or preferred pathways and there were neither tracks ahead nor trails behind them.

The basement membrane apparently functions as a filter by being a yielding substrate through which molecules beyond a certain size are normally retained as a result of physicochemical forces operating in the glycoprotein gel which forms the basement membrane in absence of pores or preformed channels. As a filter, it has no vital selectivity. It retains plasma proteins and lipids, but is penetrated by glucose, and other dissolved materials. Its barrier effect is purely physicochemical in nature depending on membrane and fluid properties, surface areas and pressure gradients. Therefore, any alteration in the chemical composition of the basement membrane or its physical state will affect its properties and consequently alter its barrier effect.

The attenuation and fenestration of the glomerular capillary endothelium can only be interpreted as a means to intensify the contact between the blood plasma and the basement membranes, and also to obviate the vital activity of the endothelial cells in this situation to as little as possible, and to facilitate the purely physical process of filtration to proceed. The glomerular endothelium possesses the widest intracellular pores which have been found in mammalian capillaries (153), and the basement membrane has been calculated to be the only barrier for 30% of the total area of the glomerular capillary surface (18). This, and the fact that the cytoplasmic portion of the fenestrated part of the endothelium virtually consists of cell membrane and a very thin layer of cytoplasmic matrix, suggests an endeavour to reduce the endothelial barrier precisely at this point to as thin a layer as possible.

It seems logical that extremely thin fenestrated endothelial layers facilitate exchange on a physicochemical basis and that comparatively thick, non fenestrated layers are able to restrict transport. However, in addition, the thicker the endothelial lining of a capillary the more is its activity of selection of transmitted materials. In muscle capillaries, the endothelium forms a continuous layer without fenestrations; and these were estimated to be a hundred- fold less permeable to water than glomerular capillaries (98). Muscle capillaries, however, show a selective protein transport as indicated by the prevalent shift of serum albumin into muscle tissue after release of a tourniquet (99). It has similarly been shown that the passage of proteins through the human placenta is selective in that the large gamma globulin passes more readily to the foetus than do the smaller albumin molecules (97). The mechanism of this active selectivity performed by the endothelial

cells is one of pinocytosis and cytopempsis (98), which is apparently lacking in the attenuated peripheral portions of the glomerular endothelial lining.

The fenestrated endothelium should be visualised as a living tissue and not simply as a sieve. By varying the number and distribution of its fenestrae or even closing them completely (Fig. 58) for a time, it can act as a possible valve by controlling the area of the filter directly exposed to the blood plasma.

The endothelial cell bodies, however, apparently still maintain the pinocytic activity inherent to all types of endothelial cells (and indeed to all animal cells). They have not infrequently been observed containing numerous vacuoles filled with a granular material and apparently being engulfed by them from the capillary lumen (Fig. 59). This was noticed more frequently in those deeply situated axial endothelial cells.

An experimental evidence to this effect has recently been presented by Farquhar et al. In experiments with colloidal gold (41), and ferritin (44) administered to young rats to determine the behaviour of particles (40-200 Å) in the glomerular capillaries, it was found that at early time points the tracer particles accummulated against the luminal surface of the basement membrane in peripheral regions of capillary loops. After longer intervals, relatively few particles were found in this location whereas large numbers were present in the spongy areas of the axial regions. There was also a striking change in the morphology of the endothelial cells, particularly in the colloidal gold experiments; for they exhibited rounded pseudopddia with complicated interdigitations of the cell membranes.

Particles were also found within the endothelial cytoplasm. These experiments suggested that the glomerular endothelium was capable of a type of microphagic activity whereby it apparently functions in the selective removal of substances which do not pass through the basement membrane. Theconcentration of filtration residues in the axial regions have been postulated to be due to "sweeping" of the luminal surface of the basement membrane by the endothelium, thus facilitating their subsequent incorporation primarily by the deeper cells of the axial region (44). This was the first suggestion that these axial endothelial cells might differ from the rest of the endothelium in function.

Attention should be drawn to the areas of contact between adjacent endothelial cells. Each endothelial cell is united around its circumference to the neighbouring cell by a belt comprised of a specialised attachment structure. These structures appear to be barriers between the capillary lumen and Bowman's space and to be bonds between adjacent endothelial cells as well. They are to be regarded as corresponding in function to the "terminal bars" of columnar epithelium, or the "desmosomes" or nodes of "Bizzozero" of the intercellular bridges of stratified squamous epithelium, or to the intercalated discs of heart muscle. They represent areas where the cell membrane is specialised, of greater density than elsewhere, and firmly attached to its opposite member. No gaps, defects, openings, pores or slits have been discerned in these attachment belts, although hundreds have been examined. All components of the attachment belt are denser than the blood plasma. Thus, no evidence for channels containing plasma passing between endothelial cells has been observed, contrary to Chambers and Zweifach's claim (19) that preferantial filtration passages might exist between endothelial cells.

The functional role of the epithelial cells is not yet very clear. The primary urine after its passage through the basement membrane must traverse the narrow clefts between the pedicels, and the pericapillary sinus in order to reach the capsular space. In this way it intimately bathes the epithelial cells. This arrangement, as well as the existence of the filtration slit membranes which has been confirmed in this study, appear to be significant. If the filtration slit membranes are only or mainly permeable to water and dissolved substances but relatively impermeable to the larger molecules of protein and lipid, the existing structural arrangement would provide maximal opportunity for the small amount of protein and lipid which normally leaks through the basement membrane to come into contact with the epithelial cell surface and be incorporated by pinocytosis. Pinocytotic activity has actually been observed in the pedicels (Fig. 27). The accumulation of pigments as trypan blue (153) and of hyaline protein droplets in clinical and experimental nephrosis (42) within the epithelial cells would argue that they try to engulf substances which have bypassed the ultrafilter, or passed it in excessive quantities.

The epithelial and the endothelial cells have relatively few intracytoplasmic organelles, and particularly few mitochondria. The mitochondria in both types of cell are small, with little internal membranes and are loosely situated in the perinuclear region without any specific arrangement. They are hardly ever seen in the pedicels and are quite few in the trabeculae. This is a significant finding, because mitochondria - as carriers of the enzyme systems involved in the energy - producing cellular reactions - provide a reliable morphological criterion, by their size, number, shape and arrangement, of the metabolic activity of a cell.

Compared with other types of mammalian cells the glomerular epithelial and endothelial cells will appear to have a relatively low metabolic activity; little activity over and above their maintenance metabolism. In other words, the glomerular epithelial and endothelial cells normally perform their function without using much energy in excess of that necessary for maintenance metabolism.

If the function of these cells is, mainly phagocytosis, as suggested here, then their morphological structure entirely agrees. Phagocytosis is a relatively primitive cell function requiring little energy. Their simple structure similarly excludes the possibility that they take an active part in glomerular filtration. This entirely agrees with the physiological evidences that the necessary filtration pressure in the glomerular capillaries is not locally generated by the glomerular cells but is provided by the systemic blood pressure, the energy of which is generated by the heart.

The presence and nature of intercapillary tissue in the renal glomerulus have been controversial points for many years. Greenfield (54), Herring (66), Kimmelstiel and Wilson (72), Zimmermann (165), Erlich (39), Bensley and Bensley (10), Goormaghtigh (52), Pak Pay (109) Sagaguchi (140) and Theones (153) considered that it did exist and was probably fibrous in nature. McManus (89), Yamada (164) and Robertson and More (136) considered that a third type of tissue was present in the glomerulus but could not stipulate its exact nature. Bell (8), Allen (1), Pease and Baker (118), Rinehart, Farquhar, Jung and Abul-Haj (134), Farquhar, Vernier and Good (43) denied its existence, while Hall (57), Mueller, Mason and Stout (101) and Bergstrand (11) stated that although cells were present, they represented the endothelium of capillaries in

other planes. It appears that the majority of workers agree that there are some cells present, which in the plane of sectioning, are exposed neither to blood nor to Bowman's space. But it is disturbing to note that these divergent opinions have been based on interpretations of essentially similar electron photomicrographs.

As presented above, the plane of sectioning can make any definitely enothelial or definitely epithelial cell appear surrounded by basement membrane and away from direct contact with the capillary lumen or the urinary space. If such planes of sectioning would not explain these appearances, where are the cells that would appear tangentially cut in sections about 60 m μ out of a spherical body 300 μ in diameter containing a large number of capillaries running in each direction? Also, the glomerulus should be regarded as a living organ, dynamic in nature with its blood channels sometimes patent and sometimes closed, as do capillaries everywhere in the body. This has been confirmed by direct illumination observations by Elias for the amphibian glomerulus (35).

As mentioned above, the final answer will be obtained only by a study of uninterrupted serial sections by the electron microscope. However, a study of the embryological development of the glomerulus is an important indirect way to answer this greatly disputed question.

THE DEVELOPMENT OF THE RENAL GLOMERULUS.

THE DEVELOPMENT OF THE RENAL GLOMERULUS.

Introduction.

The prevalent concept of glomerular development is that of invagination of a tuft of capillaries into the blind end of the developing nephron. This was first suggested by Gerlach over 100 years ago (48) and supported by Remak (125) and by Toldt (156). The invagination theory, however, did not receive unanimous support by the early investigators. Bornhaupt (13), Thayssen (151), Riedel (132), Herring (66), Huber (67), Reinhoff (124), Vimtrup (158) and Loeffler (80) denied the theory and suggested that the glomerulus developed by the ingrowth of solid cords, of primitive endothelial cells into the adjacent, still solid mass of primitive tubule cells. The end of the apposition to the invagination theory was marked by the detailed works of von Möllendorff (96) and Zimmermann (165) when the general mesangial-sling concept was introduced and remained acceptable until the advent of electron microscopy. Recently, however, the problem was again stirred and some anatomists as Edwards (32) agreed with Toldt's invagination theory, while others, as Lewis (78,79), supported Herring's findings and denied the invagination concept.

The first electron microscopic study on the development of the renal glomerulus was reported in the rat by Hall in 1954 (58). He seriously challenged the invagination hypothesis, and in a later work (61) he confirmed that the glomerulus differentiated in situ by proliferation and readjustment of its cells. Kurtz (75) reported on glomerulogenesis in the human kidney as observed by the electron microscope and, in essence, his findings were in general agreement with those reported by Hall and Roth (61). The only points of difference involved the formation of the

epithelial foot processes and the basement membrane. Suzuki (150) also observed by the electron microscope the glomerular differentiation of rats. His findings and interpretations greatly differ from those of Hall and of Kurtz. He thinks that blood cavities within the developing glomerulus have communications with cortical renal sinusoids at a very early stage of development and he believes that, what he calls glomerular anlage cells, differentiate superficially into endothelial cells and deeply into mesangial cells, thus supporting Zimmermann's concept (165) of the existence of a supporting third cell other than the epithelial and endothelial cells.

It seemed that electron microscopic studies of the immature renal glomerulus might yield information concerning its development which would be valuable to better understanding of the complicated structure of the mature glomerulus, and particularly to help in answering the disputed question of the existence of a third type of cell.

Material.

Kidneys from "Wistar" Albino rats, four days old, were used for the study. It has been shown both by Kittelson (73) and by Arataki (3) that only about one third of the number of glomeruli that are to be found in the adult rat kidney are present at birth. Glomeruli continue to be formed after birth for about 100 days; however, most of these are formed, although they remain immature for a time, in the first 3 to 4 weeks.

Results.

The earliest recognisable structure of the glomerulus is a mass of undifferentiated cells adjoining each other with no spaces in between. The primordial cell group which is destined to become the epithelial element

is then differentiated from the primitive endothelial cells by the appearance of vesicles between them separating them apart (Fig. 60), while the endothelial cells remain as a closely-packed cell mass with definite cell membranes separating them (Fig. 61). A slight change in the shape of the cell, the appearance of the nucleus and the cytoplasm differentiating these two types of primitive cells become simultaneously apparent. The primitive epithelial cell becomes moreor less cuboidal in shape, its nucleus becomes denser and its cytoplasm scantier than the primitive endothelial cell, and probably contains more mitochondria. No basement membrane can be recognised between these two types of cells at this early stage of differentiation. The vesicles separating the primitive epithelial cells then coalesce and form clefts that separate them further apart and result also in the major share of these cells to become intimately inter-related with the developing endothelial cells, while a single layer of cells remains on the opposite side of the major cleft (Fig. 62). Until this stage, the epithelial cells can be seen to show evidence of multiplication and cell division (Fig. 63).

The separated single layer of epithelial cells are then noticed to change from a cuboidal shape and gradually to become flattened. A basement membrane becomes apparent outside these cells, and together, they form Bowman's capsule and its lining cells (Fig. 64).

The capsular space then gradually enlarges and the rest of the epithelial cells get more separated. (Fig. 65). They then quickly differentiate and begin to show a large number of fine processes of the cell surface at all points where they are in relation to endothelial cells (Fig. 66). Gradually, protoplasmic extensions from these cells become noticed and the

fine foot processes become mainly derived from these primitive trabeculae (Figs. 67,68,69).

A delicate basement membrane first makes its appearance in relation to the developing foot processes when they approach the primitive endothelial cells (Fig. 67, 68, 70). It is very thin and delicate at the beginning, thinner than the basement membrane of Bowman's capsule. It can be seen outside the endothelial cell membrane, not following the membrane of individual cells, but surrounding the whole endothelial cell mass (Fig. 61, 67,68,70). However, the basement membrane is in close intimate relationship with the smooth,continuous endothelial cell mass surface membrane and does not follow the numerous folds of the epithelial cell surface membrane.

No lumen can be seen within the endothelial cell mass until differentiation of the epithelial cells is well marked; the capillary lumina are the last thing to appear in the glomerular tuft. In moderately later stages of development erythrocytes can be seen in the central mass of endothelial cells (Fig. 71). Lumina appear to begin development with the appearance of cytoplasmic vesicles within individual endothelial cells (Fig. 70). Later, as the vesicles enlarge, the endothelial cytoplasm except around nuclei becomes attenuated (Fig. 72). However, it is much thicker in the immature glomerulus than it is in the mature one, and lacks fenestration till complete development (Fig. 73).

The arterioles of the immature glomerulus have no lumina even when the appertaining glomerulus contains red blood corpuscles, and capillary lumina (Fig. 74 and 75). This contrasts with the patent capillaries in between the tubules (Fig. 74).

Discussion.

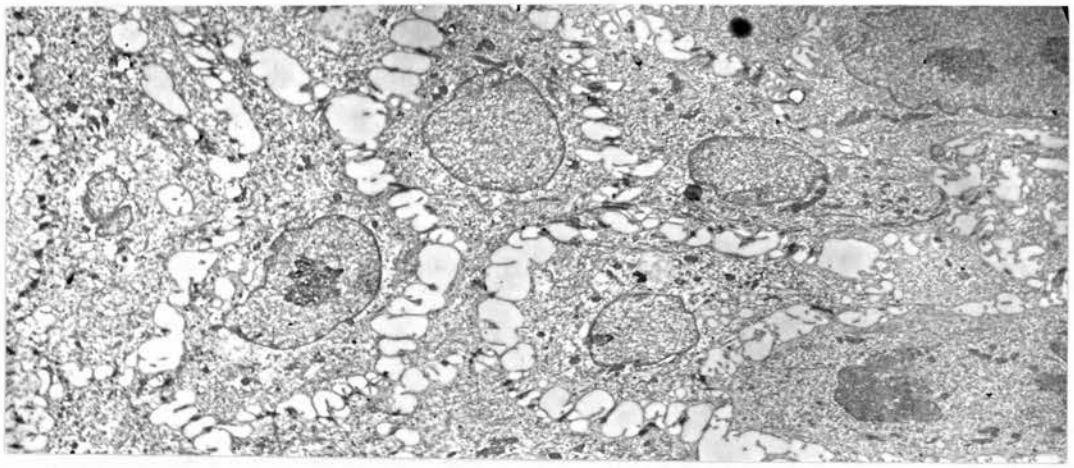


Fig. 60 Immature Rat Glomerulus: Vesicles have appeared and are separating the primitive epithelial cells apart. Note mitosis in two cells on the right side of the picture. x 9,000

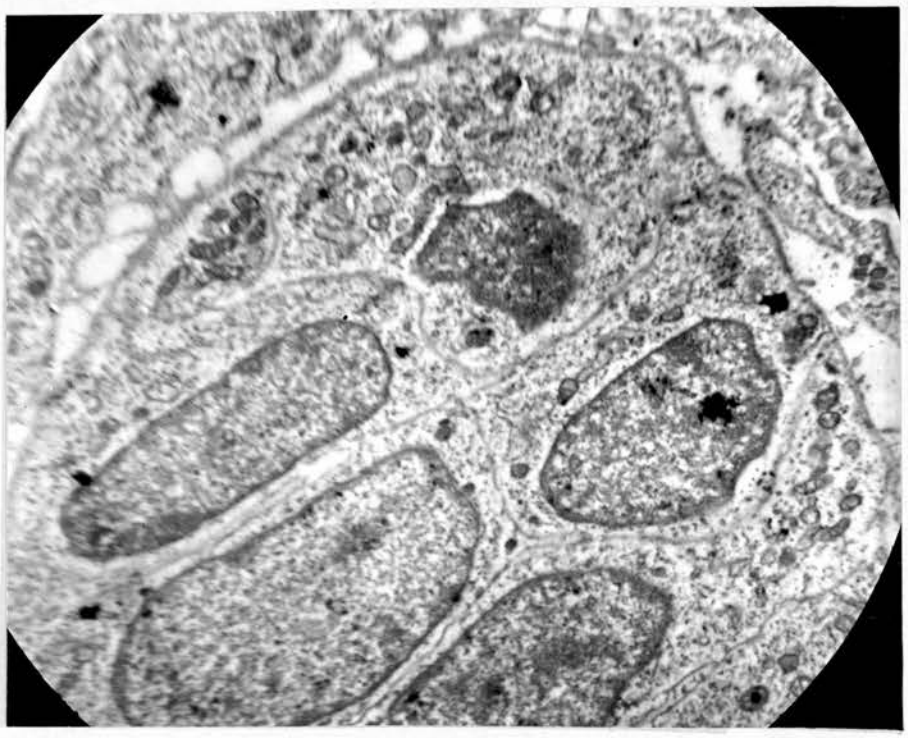


Fig. 61. Immature Rat Glomerulus: Closely-packed primitive endothelial cell masses. x 6,250

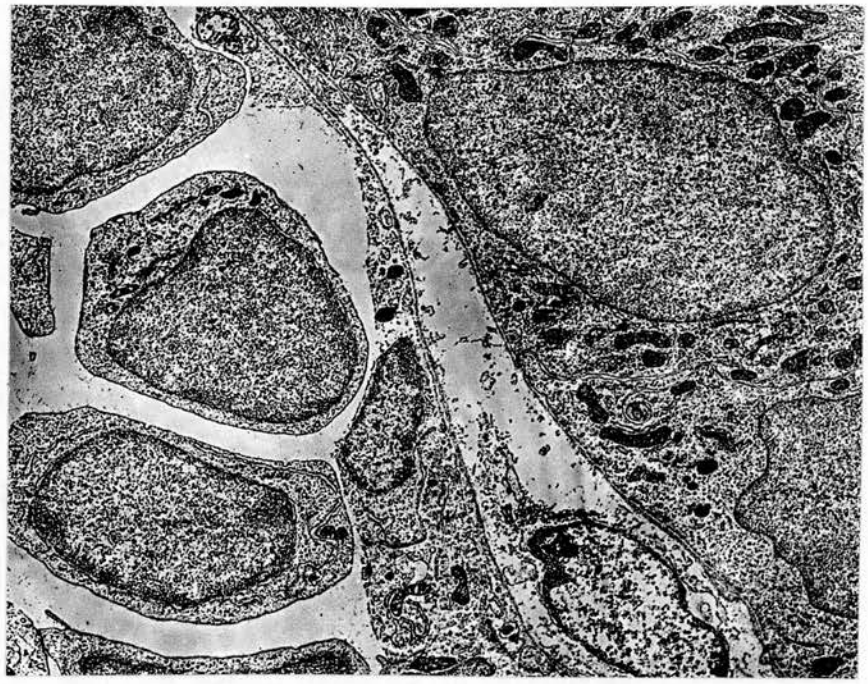


Fig. 62 Immature Rat Glomerulus: A single layer of epithelial cells has been pushed to the periphery of the developing glomerulus. A thin basement membrane is apparent outside this layer of cells. x 4,000

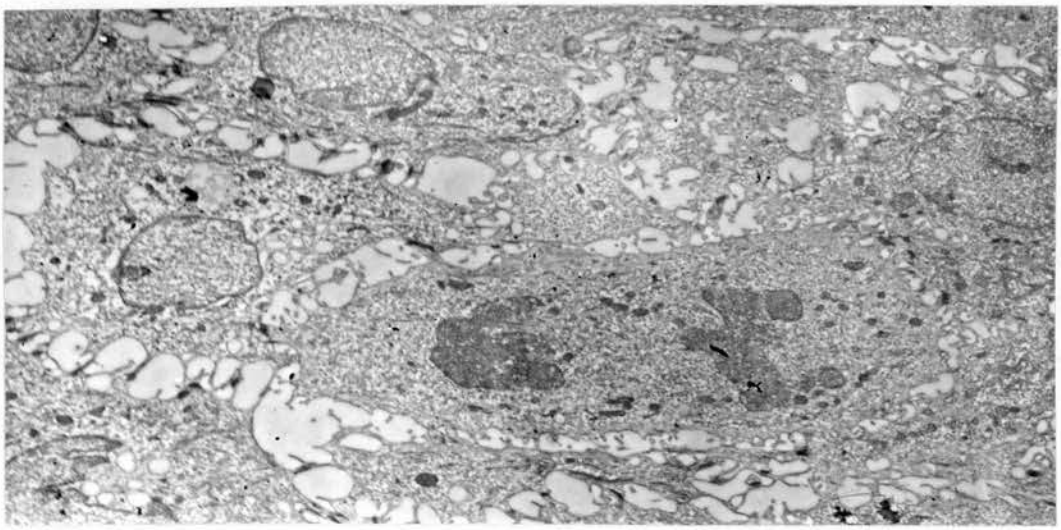


Fig. 63 Immature Rat Glomerulus: Primitive epithelial cells separating by coalescence of vesicles. Note mitosis in the cell in the middle of the field. x 9,000

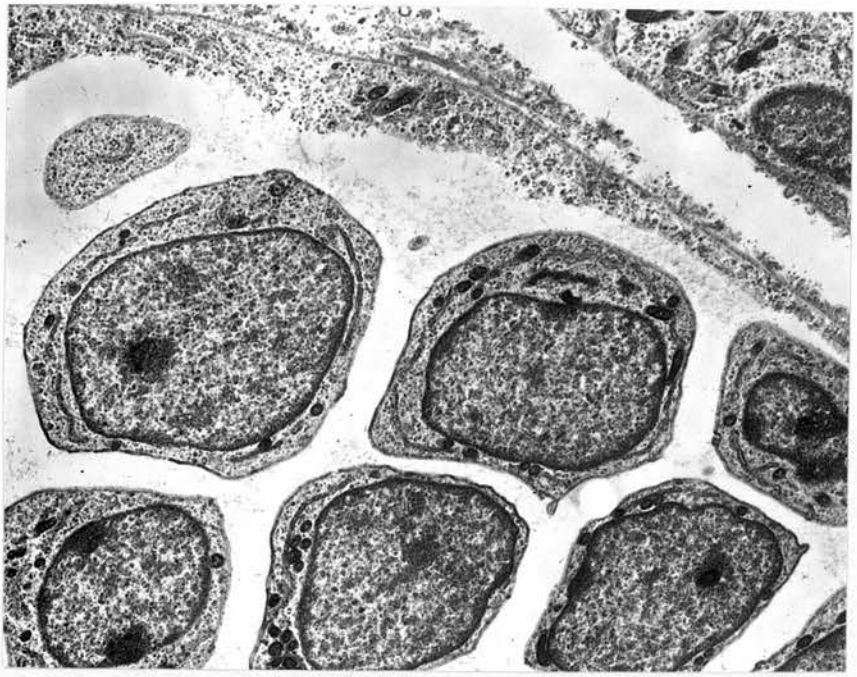


Fig. 64. Immature Rat Glomerulus: The peripheral layer of epithelial cells has become flattened and together with its basement membrane form Bowman's capsule. The rest of the epithelial cells are now cuboidal and quite separate from each other. x 4,000

Fig. 65. Immature Rat Glomerulus: The capsular space has enlarged and the cuboidal epithelial cells have become further separated apart. x 2,250

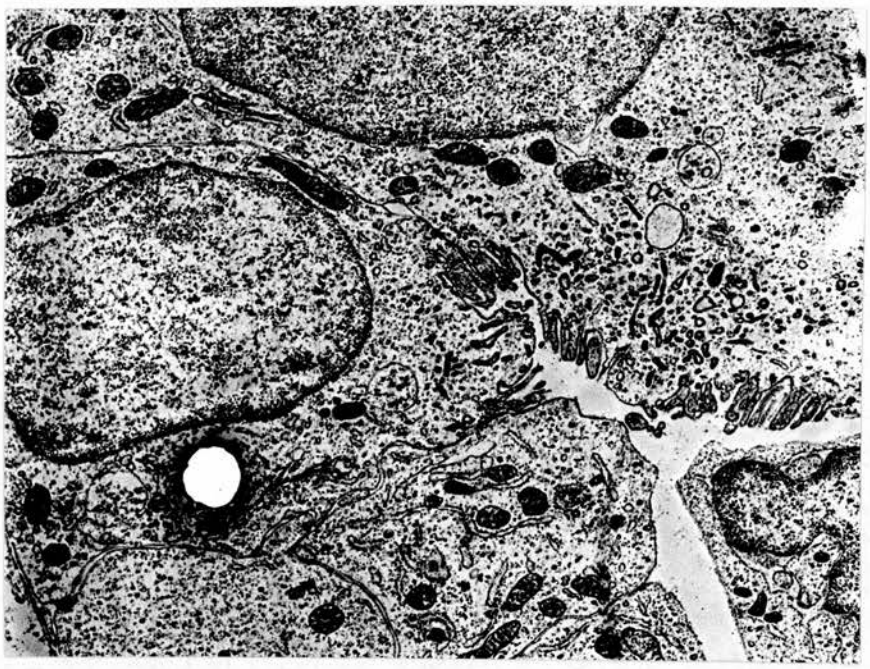


Fig. 66. Immature Rat Glomerulus: Fine protoplasmic processes are apparent on one side of a primitive epithelial cell. These are the beginning of the future pedicels. x 6,000

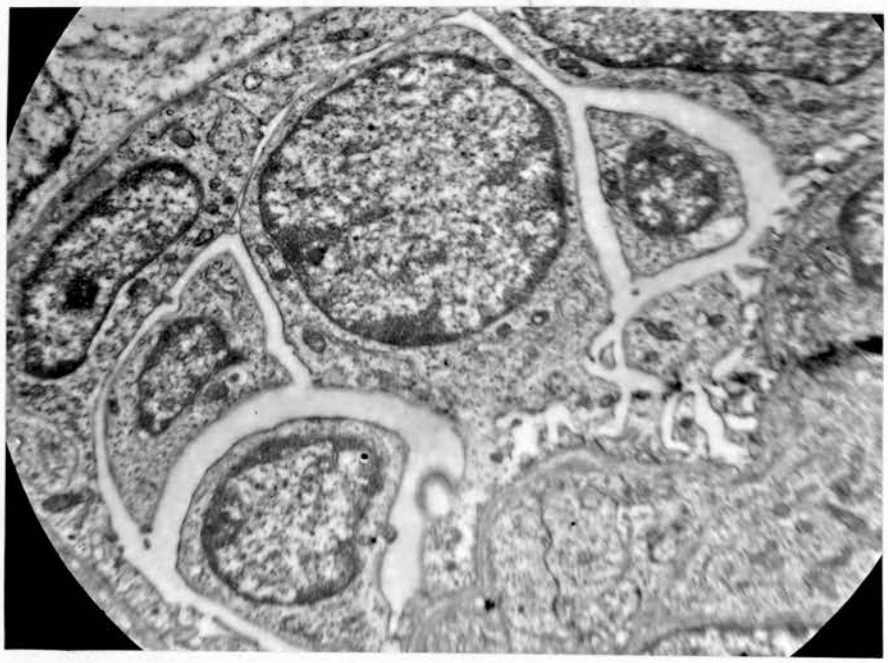


Fig. 67. Immature Rat Glomerulus: The primitive glomerular basement membrane is apparent as a thin delicate line in between the primitive pedicels and the endothelial cell mass. x 6,250

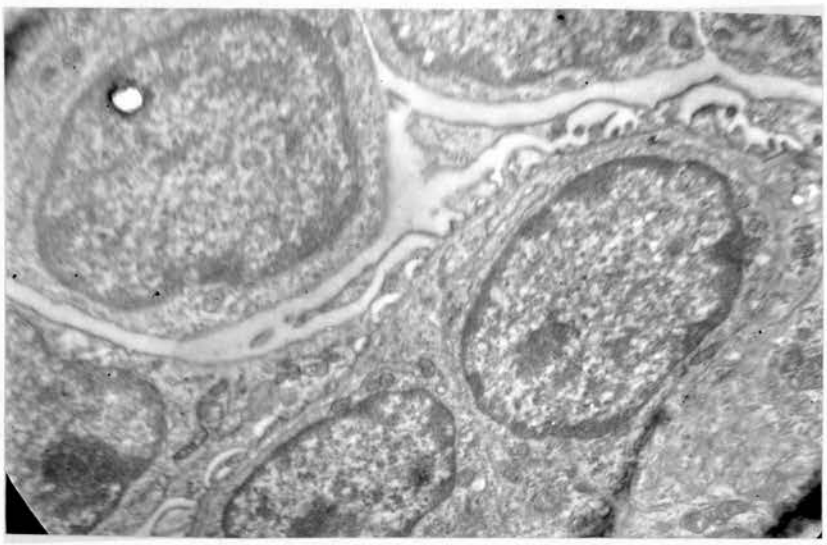


Fig. 68 Immature Rat Glomerulus: A thin and delicate basement membrane is seen outside the endothelial cell mass, intimately related to the endothelial mass cell membrane, in relation to the developing pedicels. x 6,250

Fig. 69. Immature Rat Glomerulus: The pedicels are now interdigitating with each other and are derived from protoplasmic extensions of the cell body. These will eventually become the epithelial trabeculae. x 15,000

Fig. 70. Immature Rat Glomerulus: Vacuoles: the forerunner of the glomerular capillary lumina, begin to appear within the endothelial cell mass. Note that the epithelial pedicels and the basement membrane are well developed at this stage. x 12,000

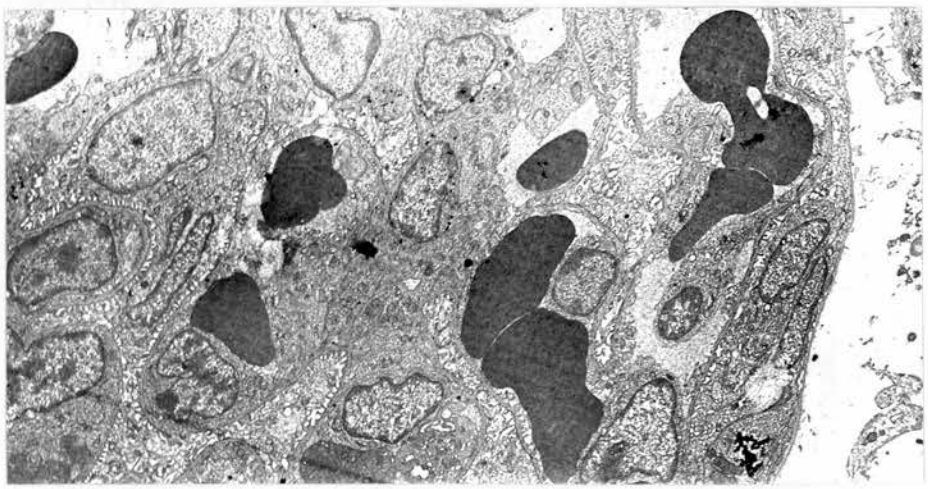


Fig. 71. Immature Rat Glomerulus: Moderately late stage of development. Many erythrocytes are seen within the capillary lumina. The glomerular arteriole of this same glomerulus was not yet patent. x 1000

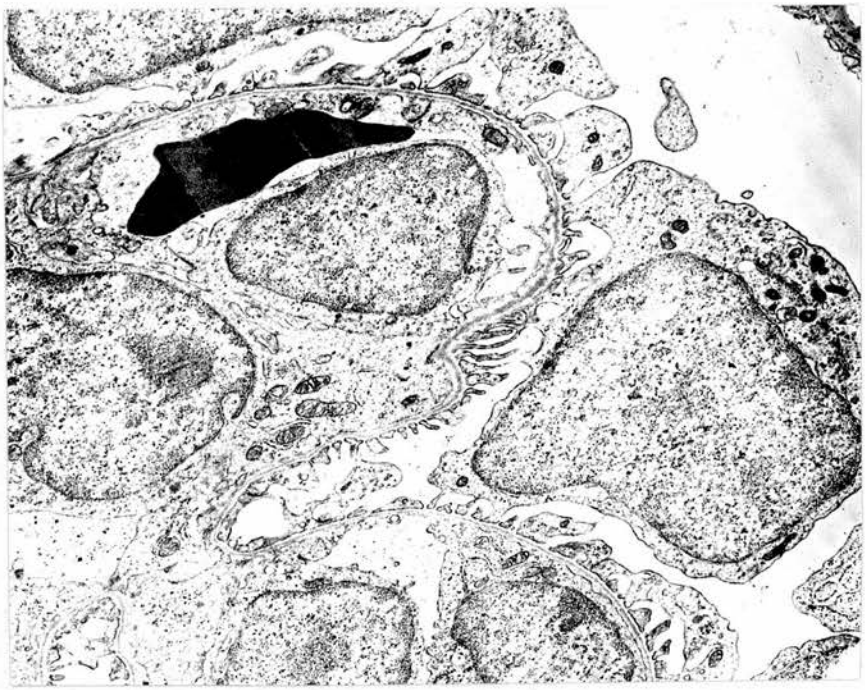


Fig. 72. Immature Rat Glomerulus: Large cytoplasmic vesicles within individual endothelial cells are apparent. The top one contains a red blood corpuscle. The appertaining glomerular arteriole was not patent. x 6,000

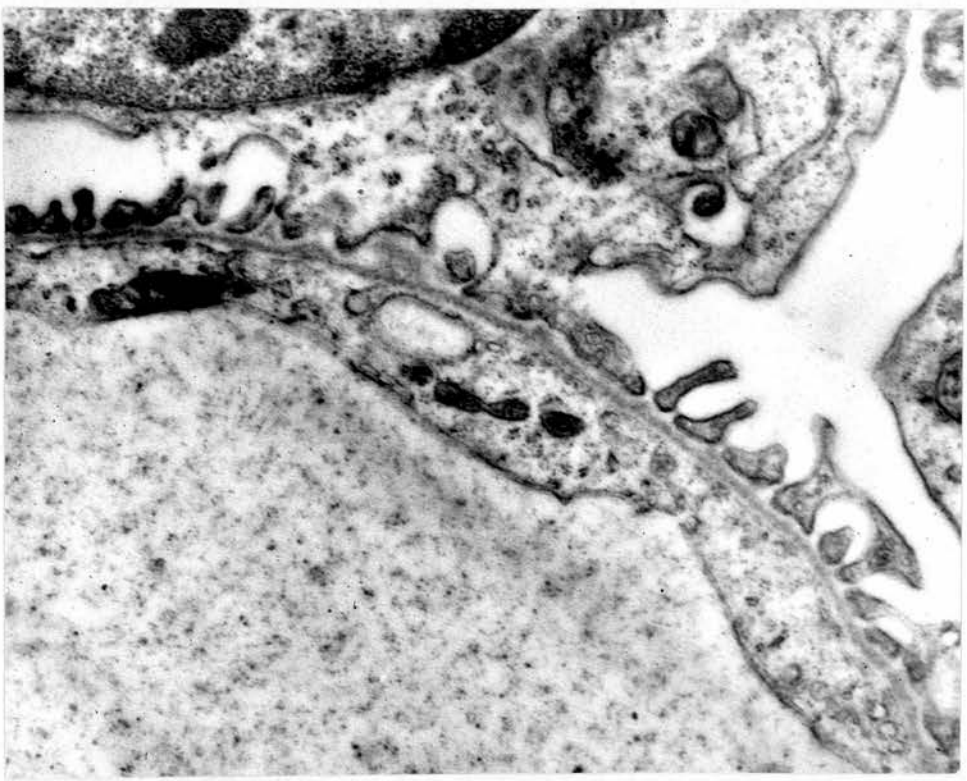


Fig. 73. Nearly mature rat glomerulus: All the structures of the capillary wall are fully developed, even the filtration slit membrane, except the fenestration of the lamina attenuata, which is apparently the last thing to occur in glomerulogenesis. x 24,000

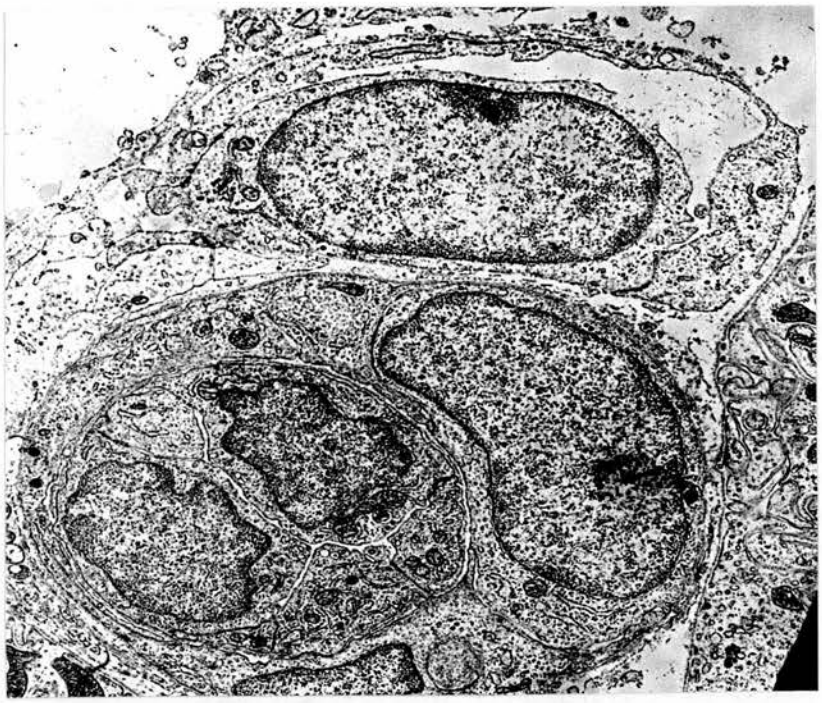


Fig. 74 Glomerular arteriole of the glomerulus seen in Fig. 72. Note that it has no lumen in contrast with the intertubular capillary seen above which is patent. x 6,000

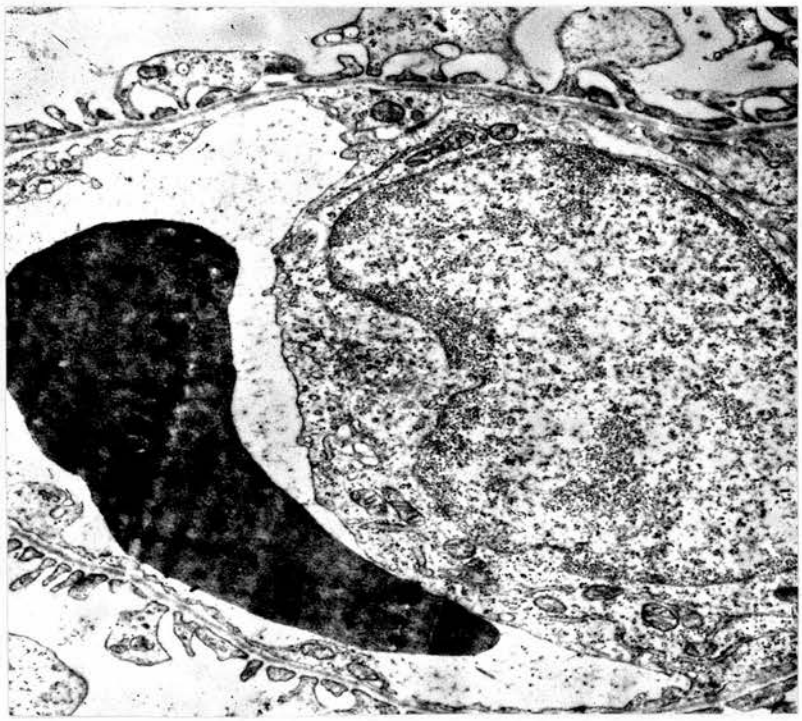


Fig. 75. Nearly mature rat glomerulus. The last thing to develop is the fenestration of the lamina attenuata. x 15,000

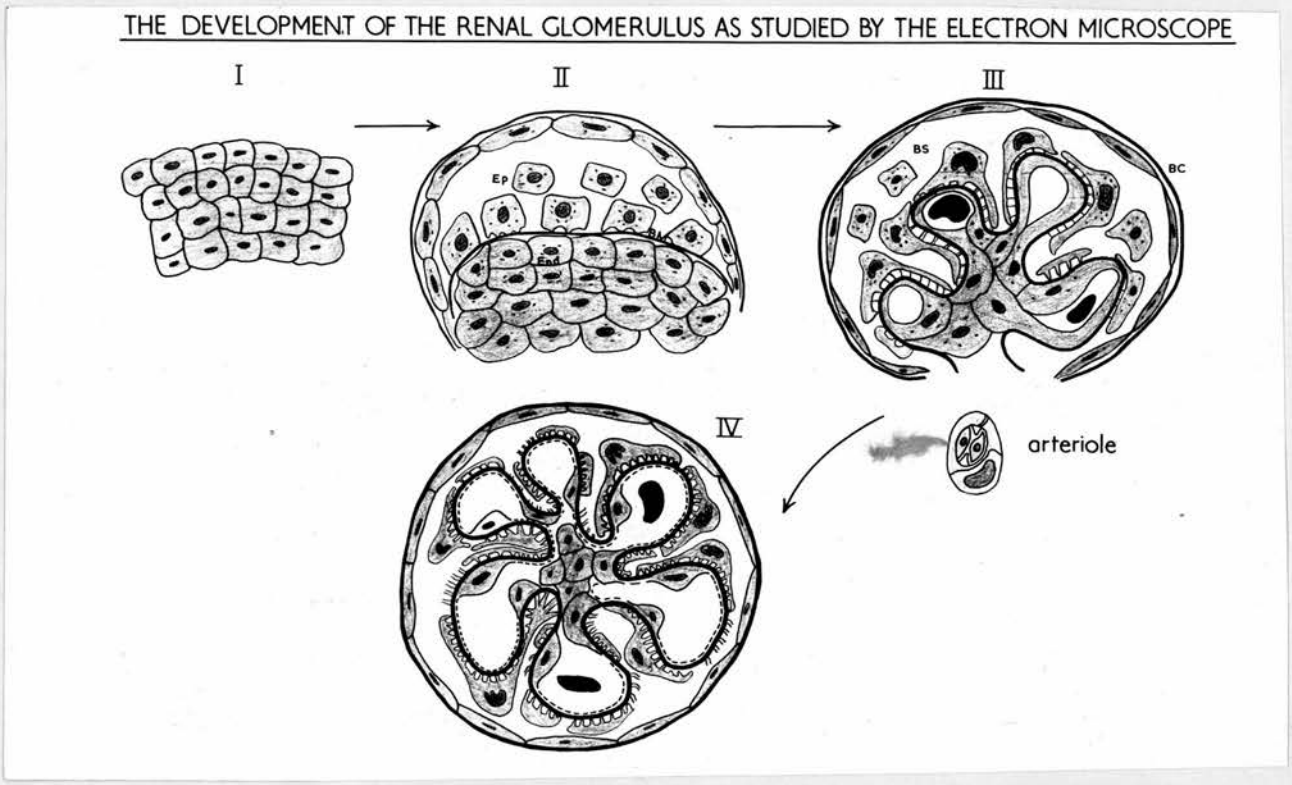

THE DEVELOPMENT OF THE RENAL GLOMERULUS AS STUDIED BY THE ELECTRON MICROSCOPE
I
II
III
Ep
End
BS
BC
IV
arteriole

Diagram 2.

Discussion.

The present observations confirm the concept that the renal glomerulus differentiates in situ by proliferation and adjustment of its cells (Diagram 2). At no time was there seen a large hollow cavity which was subsequently invaginated by developing capillaries. The basement membrane of Bowman's capsule was not present about the early corpuscle and could only be clearly defined when the intervening cleft of the capsular space has separated a parietal layer of epithelial cells. The fact that the space separating the parietal from the visceral layer of the epithelial cells once formed gradually enlarges, speaks against invagination. As Hall and Roth have pointed out (61), if invagination played a major role in glomerular development, the capsular space should decrease. Since the concept of "mesangium" is based upon the invagination of the renal corpuscle by capillary loops to form a glomerular stalk or mesentery, and since this invagination hypothesis was proved to be fallacious by this study as well as by those reported by Hall (58), and by Kurtz (75), it should no more be tenable.

Hall's observations that the epithelial cells differentiate pedicels and trabeculae long before the lumina of the capillaries become apparent or circulation established, has been confirmed. The demonstration that the basement membrane surrounds the whole endothelial cell-mass and not each individual endothelial cell greatly supports Elias's concept of glomerular structure. Elias (34) believes that a multifingered mass of cells extends into the glomerulus from its hilus; and within it there is a multitude of anastomosing channels - the capillary lumina. Overlying this mass of cells and lumina is a continuous sheet of basement membrane,

continuous at the hilus with the basement membrane of Bowman's capsule but with no basement membrane (or connective tissue elements) within this mass of cells. He ascribed to this arrangement the value of allowing the blood channels within the glomerulus to shift from one place to another.

Hall (58) attributed to the endothelial cells the function of formation of the basement membrane. Farquhar et al (44) thought that both the endothelial and epithelial cells contribute to the formation of the basement membrane. Kurtz (75) observed that the primitive basement membrane consisted of two layers and suggested that each layer represented a contribution from epithelial and endothelial cells. In the electron micrographs reported in this study, it can be clearly seen that the basement membrane consists of but one thin delicate membrane related to the developing foot processes outside the endothelial cell membrane and close to it. The suggestion of a double structure in Kurtz's electron micrographs is probably due to artefact as his material was fixed within one hour after death. Similarly, his observation that the endothelial cell mass is syncytial,is not true, as definite cell membranes were observed between endothelial cells and persisted as the endothelial attachment belts in the mature glomerulus even when the bulk of the cytoplasm has been attenuated (Fig. 10 and 11). Since every capillary in the fully developed glomerulus is surrounded by a basement membrane, at first thought it might be assumed that these basement membranes are produced by the endothelial cells of the capillaries. However, some capillaries in other parts of the body are not covered by basement membrane (62). Furthermore, pronounced basement membranes are found around all the epithelial tubules of the kidney between their epithelial walls and the connective tissue stroma in which they lie. The fact that the basement membrane

was always related to the epithelial foot processes as they developed and came in contact with the surface of the endothelial cell mass, as well as the appearance of a basement membrane around the parietal layer of epithelial cells as they are separated from the bulk of the epithelial cells leads to the conclusion that the basement membrane seen around the capillaries in the glomerular tuft must have formed under the influence of epithelial cells. On the other hand, the close intimate relationship of the developing basement membrane with the endothelial cell mass surface membrane, and the fact that the basement membrane does not follow the numerous folds of the epithelial cell surface membrane is significant. It appears that the glomerular capillary basement membrane forms under the influence of both epithelial and endothelial cells where their cell surface membranes come in contact with each other.

This conclusion must inevitably lead to the further conclusion that the visceral epithelial cells must thoroughly permeate through the interstices that exist in the adult glomerular tuft to come into intimate contact with the surface of all capillaries. Everywhere throughout the glomerulus the epithelial cells apply themselves to the surfaces of capillaries to provide them with basement membrane. Hall and Roth (61) have suggested that the epithelial cells by sending their trabeculae and pedicels to permeate deeply into the primitive endothelial cells, play a role in the formation processes which organise the capillaries into their characteristic pattern.

The presence of erythrocytes within primitive endothelial cells was reported by early investigators (66, 107) as well as by Hall and Roth (61) and by Kurtz (75) before circulation has been established. Rienhoff (124)

observed that embryonic chick metanephros in tissue culture grew epithelial cells, endothelial cells and blood cells. This might represent some totipotent quality of the primitive cell mass destined to form the renal corpuscle as suggested by Okkels thirty years ago. The fact that erythrocytes were seen within glomeruli whose arteriole was not patent yet, indicates that they are developed locally from the primitive endothelial cells and not as Suzuki suggested that they indicate that communication of the blood cavities within the glomerulus with the sinusoids formed in the renal cortex exist from its early stages.

REFERENCES.

1. Allen, A.C.
The Kidney: Medical and Surgical Diseases.
Grune and Stratton, Inc., New York, 1951.

2. Andrejewitsch, A.T.
Über das Epithel der Sammelröhren in der Saugerniere.
Medical Dissertation. Bern, 1919. Quoted by Möllendorff (96)

3. Arataki, M.
On the postnatal growth of the kidney, with special reference to the number and size of the glomeruli.
Am. J. Anat., 36: 399, 1926.

4. Aschoff, L.
Uber die Blutbildung in der Niere.
Schweiz Med. Wchnschr., 65: 193, 1935.

5. Bargmann, W.
Weitere histologische Untersuchungen an Nierenkörperchen.
Ztschr. f. Zellforsch, u. mikr. Anat., 18, 166, 1933.

6. Bargmann, A., Knoop, A., Schiebler, T.H.
Histologische cytochemische und electronenmikroskopische untersuchungen am nephron. (Mit Berücksichtigung der Mitochondrien).
Ztschr. f. Zellforsch. u. mikro. Anat., 42, 386, 1955.

7. Bayliss, L.E., Kerridge, M.T. and Russell, D.S.
The excretion of protein by the mammalian kidney.
J. Physiol., 77, 386, 1933.

8. Bell, E.T.
Renal Diseases. 2nd edition.
Lea and Febiger, Philadelphia, 1950.

9. Bensley, R.D.
Am. J. Anat., 44, 141, 1929.

10. Bensley, R.R. and Bensley, R.D.
The structure of the renal corpuscle.
Anat. Rec., 47, 147, 1930.

11. Bergstrand, A.
Electron microscopic investigations of the renal glomeruli.
Lab. Invest., 6, 191, 1957.

12. Bohle, A. and Krecke, H.J.
Zur Frage der Basalmembrenen der Glomerulumschlingen in der Niere des Menschen.
Virchow's Arch. path. Anat., 327, 663, 1955.

13. Bornhaupt, (1867),
Quoted by Herring (66).

14. Bott, P.A. and Richards, A.N.
The passage of Protein molecules through the glomerular membranes.
J. Biol. Chem., 141; 391, 1941.

15. Bowman, W.
On the structure and use of the Malpighian bodies of the kidney, with observations on circulation through that gland.
Philos. Tr. Roy. Soc., London, 132: 57, 1842.
Reprinted in Medical Classics, 5: 249, 1940-1941.

16. Boyer, C.C.
The vascular pattern of the renal glomerulus as revealed by plastic reconstruction from Serial Sections.
Anat. Rec., 125: 433, 1956.

17. von Breeman, V.L., Reber, J.F. and Cooper, W.G.
J. Biophys & Biochem. Cytol., 2: (Suppl), 283, 1956.

18. Brown, R. and Danielli, J.F.
Active Transport and Secretion.
Academic Press Inc., 1954, New York.

19. Buday, K.
Experimentell-histologische Studien über die Genese des Nierentuberkels.
Virchow's Arch. Path. Anat., 186: 145, 1906.

19a. Chambers, R. and Zweifach.
Intercellular cement and capillary Permeability.
Physiol. Rev., 27: 436, 1947.

20. Chinard, F.P.
Possible mechanisms of formation of glomerular fluid.
Tr. Conf. Renal Function, Josiah Macy, Jr., Foundation, New York, 1951

21. Chinard, F.P., Vosburgh, G.L. and Enns, T.
Transcapillary exchange of water and other substances in certain organs of the dog.
Am. J. Physiol., 183: 221, 1955.

22. Clara, M.
Vergleichende Histobiologie des Nierenglomerulus und der Lungenalveole (Nach Untersuchungen bein Menschen und beine Kaninchen).
Ztschr. mikr. anat. Forsch., 40; 147, 1936.

23. Clark, S.L.
Cellular differentiation in the kidneys of newborn mice studied with the electron microscope.
J. Biophys. & Biochem. Cytol., 3: 349, 1957.

24. Cook, W.F. and Pickering, G.W.
The location of renin within the kidney.
J. Physiol., 143: 78, 1158

25. Corner, G.W.
On the widespread occurrence of reticular fibrils produced by capillary endothelium.
Carnegie Institute Contrib. Embryol. 1920, 9, no. 272, pp. 85-93, 7 pl.

26. Crabtree, C.E.
Influence of pregnancy on renal cortex of mice as indicated by structure of Bowman's capsule.
Endocrinology, 29: 204, 1941.

27. Cushny, A.R.
The Secretion of the Urine, 2nd Ed.
Longmans, Green & Co., London, 1926.

28. Dalton, A.J.
Structural details of some of the epithelial cell types in the kidney of the mouse as revealed by the electron microscope.
J. Nat. Cancer Inst., 11: 1163, 1951.

29. Dalton, A.J.
A chrome-osmium fixative for electron microscopy.
Anat. Rec., 121: 281, 1955.

30. De Muylder, C.G.
The "neurility" of the kidney. 1952, Oxford.
Blackwell Scientific Publications, London pp. 1-41.

31. von Ebner, V.
Koelliker's Handbuch der Gewebelehre des Menschen.
6th Ed., Vol., 3, W. Engelmann, Leipzig, 1902, p. 1020.

32. Edwards, J.G.
The development of the efferent arteriole in the human metanephros.
Anat. Rec., 109: 495, 1951.

33. Elias, H.
The renal glomerulus by light and electron microscopy.
Res. Serv. Med., 46: 3, 1956.

34. Elias, H.
De Structura Glomeruli Renalis.
Anat. Anzeig., 104; 26, 1957.

35. Elias, H., Hoasmann, A., Barth, I.B., and Solmor, A.
Blood flow in the renal glomerulus.
J. Urol., 83; 790, 1960.

36. El-Mahallawy, M.N. and Sabour, M.S.
Etiological factors in diabetic nephropathy.
J. Am. Med. Ass., 173: 1783, 1960.

37. El-Mahallawy, M.N., Sabour, M.S. and Osman, L.M.
Clinical and diagnostic aspects of diabetic nephropathy.
Scott. Med. J., 5, 335, 1960.

38. El-Mahallawy, M.N., Sabour, M.S., Osman, L.M. and Sadek, S.H.
Metabolic and endocrine aspects of diabetic nephropathy.
Brit. Med. J., 1: 674, 1960.

39. Erlich, W.
Morphologic differentiation of nephritis in the rat and the therapeutic effects of anticoagulants and proteolytic enzymes.
In: III Experimental Nephrotic Syndrome.
Proc. 5th Ann. Conf. Nephrotic Syndrome, Philadelphia, Nov. 5-7, 1953. National Nephrosis Foundation, New York, 1954, p. 117-134.

40. Farquhar, M.G., Hopper, J.Jr., and Moon, H.D.
Diabetic Glomerulosclerosis: Electron and light microscopic studies.
Am. J. Path., 35: 721, 1959.

41. Farquhar, M.G. and Palade, G.E.
Behaviour of Colloidal particles in the glomerulus.
Anat. Rec., 133; 378, 1959.

42. Farquhar, M.G., and Palade, G.E.
Segregation of ferritin in glomerular protein absorption droplets.
J. Biophys. & Biochem. Cytol., 7: 297, 1960.

43. Farquhar, M.G., Vernier, R.L., and Good, R.A.
Studies on familial nephrosis, II: Glomerular changes observed with the electron microscope.
Am. J. Path., 33; 971, 1957.

44. Farquhar, M.G., Wissig, S.L. and Palade, G.E.
Glomerular permeability I: Ferritin transfer across the normal glomerular capillary wall.
J. Exper. Med., 113: 47, 1961.

45. Fawcett, D.
The Submicroscopic structure of the kidney in Rana Pipiens.
Anat. Rec., 120: 733, 1954 (Abstract).

46. Gautier, A., Bernhard, W. and Oberling, C.
Sur l'existence d'un apparell lacunaire péri-capillaire du glomerule due Malpighi révéleé par le microscope electronique.
Societe de Biologie, Paris, Comptes Rendues des Seances, 144: 1605, 1950.

47. Gerlach, J.
Beiträge zur strukturhehre der Niere.
Arch. f. Anat. Physiol. u. Wissensch. Med., p. 378, 1845.

48. Gerlach, J.
Zur Anatomie der Niere.
Arch. f. Anat. Physiol. u. Wissensch. Med., p. 102, 1848.

49. Gersh, I. and Catchpole, H.R.
The organisation of ground substances and basement membrane and its significance in tissue injury, disease and growth.
Am. J. Anat., 85: 457, 1949.

50. Goormaghtigh, N.
Les segments neuromyoarterials juxtaglomerulaire du vein.
Arch. Biol., 43: 575, 1932.

51. Goormaghtigh, N.
La fonction endocrine des arterioles renales; son role dans la pathologie de l'hypertension arterielle, 1944, Fonteyn, Louvein.

52. Goormaghtigh, N.
The renal arteriolar changes in the anuric crush syndrome.
Am. J. Path., 23: 513, 1947.

53. Goormaghtigh, N.
Le mesangium du floculus glomerulaire. Ses reactions dans la glomerulonephrite aigue et les nephrites hypertensives.
J. d'Urol., 57: 569, 1951.

54. Greenfield, W.S.
Granular contracted kidney.
Tr. Path. Soc., London, 31: 157, 1879-1880.

55. Grotte, G., Knutson, R.C. and Bollman, J.L.
The diffusion of dextrans of different molecular sizes to lymph and urine.
J. Lab. & Clin. Med., 38: 577, 1951.

56. Haddy, F.J., Scott, J., Fleishman, M. and Emanuel, D.
Effect of change in flow rate upon renal vascular resistance.
Am. J. Physiol., 195: 111, 1958.

57. Hall, B.V.
Studies of Normal glomerular structure by electron microscopy.
Proc. 5th Ann. Conf. Nephrotic Syndrome, Philadelphia, Nov. 5-7, 1953. National Nephrosis Foundation, New York, 1954, p. 1-39.

58. Hall, B.V.
Further studies of the normal structure of the renal glomerulus.
Proc. 6th Ann. Conf. Nephrotic Syndrome, Cleveland, Nov. 5-6, 1954. National Nephrosis Foundation, New York, 1955, p. 1-39.

59. Hall, B.V.
in discussion of Symposium on "Histochemistry and Elucidation of Kidney Structure and Function."
held at the Jefferson Medical College, Philadelphia, April 5th,1955.
J. Histochem., 3, 310, 1955.

60. Hall, B.V.
The protoplasmic basis of glomerular ultrafiltration.
Am. Heart J., 54: 1, 1957.

61. Hall, B.V. and Roth, L.E.
Preliminary studies on the development and differentiation of cells and structures of the renal corpuscle.
Proc. Stockholm Conf. on Electron Microscopy, Stockholm, Sept. 1956.
Almquist A. Wiksell, Stockholm 1957, pp. 176-179.
Academic Press Inc. Publishers, New York, N.Y. Editors: F.S. Shostrand and J. Rhodin.

62. Ham, A.W.
Histology. 3rd Ed. Pitman Medical Publishing Co. Ltd., London, 1957.

63. Hartroft, P.M.
A preliminary study of the electron microscopy of renal juxtaglomerular cells; correlation with light microscopy.
Anat. Rec., 124: 458, 1956. Abstract.

64. Hartroft, P.M.
The juxtaglomerular cells and their influence on sodium metabolism.
Presented at the Hahremann Symposium on Edema, Philadelphia, December, 7, 1959.

65. Hartroft, P.M. and Hartroft, W.S.
Studies on renal juxtaglomerular cells I: variations produced by sodium chloride and desoxycorticosterone acetate.
J. Exper. Med., 97: 415, 1953.

66. Herring, R.
The development of the Malpighian bodies of the kidney and its relation to pathological changes which occur in them.
J. Path. Bact., 6: 459, 1900.

67. Huber, G. Carl.,
"The morphology and structure of the mammalian renal tubule."
The Harvey Lectures, 5, 100,1909-10.

68. Johnston, W.B.
A reconstruction of a glomerulus of the human kidney.
Anat. Anzeig, 16: 260, 1899.

69. Jones, D.B.
Inflammation and repair of the glomerulus.
Am. J. Path., 27: 991, 1951.

70. Jones, D.B.
Glomerulonephritis.
Am. J. Path., 29: 33, 1953.

71. Jones, D.B.
Nephrotic glomerulonephritis.
Am. J. Path., 33: 313, 1957.

72. Kimmelstiel, P. and Wilson, C.
Intercapillary lesions in the glomeruli of the kidney.
Am. J. Path., 12: 83, 1936.

73. Kittelson, J.A.
The postnatal growth of the kidney of the albino rat, with observations on an adult human kidney.
Anat. Rec., 17; 385, 1917.

74. Kulenkampff, H.
Funkionelle Veronderungen an den Deckzellen der Glomeruluscapillaren der Katzenniere.
Ztschr. Anat., 117: 520, 1954.

75. Kurtz, S.M.
The electron microscopy of the developing human renal glomerulus.
Exper. Cell Research, 14: 355, 1958.

76. Kurtz, S.M. and McManus, J.F.A.
A reconsideration of the development, structure and disease of the human renal glomerulus.
Am. Heart J., 58: 357, 1959.

77. Lapp, L.
Elektronenmikroskopische Untersuchengen zur Funktion des Nierenmarkes der Ratte.
Verh. Deutsch. Gesellsch. Für Path., 44: 294, 1960.

78. Lewis, O.J.
The development of the blood vessels of the metanephros.
J. Anat., 92: 84, 1958.

79. Lewis, O.J.
The vascular arrangement of the mammalian renal glomerulus as revealed by a study of its development.
J. Anat., 92: 433, 1958.

80. Loeffler, L.
Über den Anfand der Harnkanalchen und ihre Beziehung zum Glomerulus.
Ztschr. f. Urol., 27: 433, 1933.

81. Ludwig, C.
Handwörterbuch der Physiologie II.
Braunsweig, 1844.

82. MacCullum, W.G.
Glomerular changes in nephrosis.
Bull. Johns Hopkins Hosp., 55: 416, 1934.

83. MacDonald, M.K.
The nephrotic syndrome. Electron microscopy of the kidney.
In: Symposium, Some Aspects of Renal Disease, Royal College of Physicians of Edinburgh, March 25th, 1961.

84. MacDonald, M.K., Lambie, A.T. and Robson, J.S.
Resolution of glomerular lesions in the nephrotic syndrome treated with cortisone: Electron microscopic sutdies in an adult case.
Scott. Med. J., 4: 415, 1959.

85. McGregor, L.
The finer histology of the normal glomerulus.
Am. J. Path., 5: 545, 1929.

86. McGregor, L.
Cytological changes occurring in the glomerulus of chronic glomerulonephritis.
Am. J. Path., 5: 559, 1929.

87. McManus, J.F.A.
Further observations on the glomerular root of the vertebrate kidney.
Quart. J. Micr. Sc., 88: 39, 1947.

88. McManus, J.F.A.
Periodic acid routine applied to the kidney.
Am. J. Path., 24: 643,1948.

89. McManus, J.F.A.
Structure of the glomerulus of the human kidney.
Am. J. Path., 24: 1259, 1948.

90. McManus, J.F.A.
Medical Diseases of the Kidney.
Lea and Febiger, Philadelphia, 1950.

91. Mall, F.P.
Note on the basement membranes of the tubules of the kidneys.
Bull. Johns Hopkins Hosp., 12: 133, 1901.

92. Malpighi, M.
De Viscerum Structura, 1669, "Quoted by Bowman, 1842."

93. Marshall, M.E. and Deutch, H.F.
Clearances of some proteins by the dog kidney.
Am. J. Physiol., 163: 461, 1950.

94. Miller, F. and Bohle, A.
Electron microscopy of the glomerular basement membrane in experimental amyloidosis of the mouse.
Proc. Stockholm Conf. on Electron Microscopy, Stockholm, Sept. 1956, pp. 256-258. Almquist A. Wiksell, Stockholm, 1957.
Academic Press Inc. Publishers, New York, N.Y. Editors: F.S. Sjöstrand and J. Rhodin.

95. von Möllendorff, W.
Einige Beobachtungen über den Aufbau des Nierenglomerulus.
Ztschr. f. Zellforsch. u. mikr. Anat., 6: 441, 1927.

96. von Möllendorff, W.
Handbuch der mikroskopischen Anatomie. Teil 7[1], J. Springer, Berlin, 1930.

97. Moore, D.H., Martin du Pan, P. and Buxton, C.L.
Am. J. Abst. and Gynecol., 57: 312, 1949.

98. Moore, D.H. and Ruska, H.
The fine structure of capillaries and small arteries.
J. Biophysic. & Biochem. Cytol., 3: 457, 1957.

99. Moore, D.H. and Worf, D.L.
Effect of temperature on the transfer of serum proteins into tissues injured by tourniquet and by scald.
Am. J. Physiol., 170: 616, 1952.

100. Mueller, C.B.
The Structure of the renal glomerulus (Review).
Am. Heart J., 55: 304, 1958.

101. Mueller, C.B., Mason, A.D. Jr., and Stout, D.G.
Anatomy of the glomerulus.
Am. J. Med., 18: 267, 1955.

102. Niessing, K., and Rollhäuser, H.
Über den Submikroskopischen ban des Grundhäutchens der Himkapillaren.
Z. Zellforsch., 39: 431, 1954.

103. Nussbaum, M.
Ueber den Bau und die Thätigkeit der Drüsen. V. Mitteilung Zur Kenntniss der Nierenorgane.
Arch. f. mikr. Anat., 27: 442, 1886.

104. Oberling, C.
L'existence d'une housse neuromusculaire an niveau des arteres glomerulaires de l'homme.
C.R. Academie Sciences, Paris, 184: 1200, 1927.

105. Oberling, C., Gautier, A. and Bernhard, W.
La structure des capillaires glomerulaires vue an microscope electronique.
Presse med., 59: 938, 1951.

106. Oberling, C. and Hatt, P.Y.
Etude de l'appareil juxtaglomerulaire due Rat au microscope electronique.
Ann. Anat. Path., 5: 441, 1960.

107. Okkels, H.
Sur le development du floccule glomerulaire dans le rein foetal. Remarque sur le travail de R. Noel et H. Pigeaud.
Bull. histol. appl. physiol. et pathol. et tech. microscop., 10: 12, 1933.

108. Oliver, J., MacDowell, M., Welt, L.G., Holliday, M.A., Hollander, W. Jr., Winters, R.W., Williams, T.F. and Segar, W.E.
The renal lesions of electrolyte imbalance I: The structural alterations in potassium depleted rats.
J. Exper. Med., 106: 563, 1957.

109. Pak Poy, R.K.F.
Electron microscopy of the mammalian renal glomerulus: The problems of intercapillary tissue and the capillary loop basement membrane.
Am. J. Path., 34: 885, 1958.

110. Palade, G.E.
A Study of fixation for electron microscopy.
J. Exp. Med., 95: 285, 1952.

111. Palade, G.E.
Fine structure of blood capillaries.
J. Appl. Physics, 24: 1424, 1953.

112. Pappenheimer, J.R.
The passage of substances across capillary walls.
Tr. Conf. Renal Function. Josiah P. Macy, Jr., Foundation, New York, 1951, p. 36.

113. Pappenheimer, J.R.
Passage of molecules through capillary walls.
Physiol. Rev., 33, 387, 1953.

114. Pappenheimer, J.R., Renkin, E.M. and Borrero, L.M.
Filtration, diffusion and molecular seiving through peripheral capillary membranes: A contribution to the pore theory of capillary permeability.
Am. J. Physiol., 167: 13, 1951.

115. Pease, D.C.
Electron microscopy of the vascular bed of the kidney cortex.
Anat. Rec., 121:701, 1955.

116. Pease, D.C.
Fine structures of the kidney seen by electron microscopy.
J. Histochem. & Cytochem., 3: 295, 1955.

117. Pease, D.C.
Histological techniques for electron microscopy.
Academic Press Inc., London, 1960.

118. Pease, D.C. and Baker, R.F.
Electron microscopy of the kidney.
Am. J. Anat., 87: 349, 1950.

119. Peter, K.
Die Nierenkanälchen des Menschen und einiger Säugetiere.
Anat. Anzeig., Jena, 30: Erg. H. 114, 1907.

120. Piel, C.F., Dong, L., Modern, F.W.S., Goodman, J.R. and Moore, R.
The glomerulus in experimental renal disease in rats as observed by light and electron microscopy.
J. Exp. Med., 102: 573, 1955.

121. Policard, A.
Precis d'histologie Physiologique.
5th Edition. Paris, 1950, p. 542.

122. Policard, A., Collet, A. and Giltaire-Relyte, L.
Recherches au microscope electronique sur la structure du glomerule renal des mammiferes.
Arch. Anat. Mikr., 44: 1, 1955.

123. Porter, K.R. and Blum, J.
A study in microtomy for electron microscopy.
Anat. Rec., 117: 685, 1953.

124. Reinhoff, W.F.
Development and growth of the mesonephros or permanent kidney in chick embryos.
Bull. Johns Hopkins Hosp., 33: 392, 192.

125. Remak, R.
Untersuchungen über die Entwickelung des Winbelthieres G. Reimer, Berlin, 1851 (1855) p. 194.

126. Rhodin, J.
Correlation of ultrastructural organisation and function in normal and experimentally changed proximal convoluted tubule cells of the mouse kidney.
Thesis: Stockholm, 1954, Karolinska Institutet.

127. Rhodin, J.
Electron microscopy of the glomerular capillary wall.
Exper. Cell Research, 8: 572, 1955.

128. Rhodin, J.
Further studies on the nephron ultrastructure in mouse. Terminal part of proximal convolution.
Proc. Stockholm Conf. on Electron Microscopy, Stockholm, Sept. 1956. Almquist A. Wiksell, Stockholm 1957. Academic Press Inc., Publishers, New York, N.Y. Editors: F.S. Sjostrand and J. Rhodin.

129. Rhodin, J.
Anatomy of kidney tubules.
Internat. Rev. Cytol., 7, 485, 1958.
Academic Press Inc., New York, N.Y., 1958.
Editors: G.H. Bourne and J.F. Danielli.

130. Rhodin, J.
Electron Microscopy of the kidney.
Am. J. Med., 24: 661, 1958.

131. Ried, R.T.W.
Electron microscopy of glomeruli in nephrotoxic serum nephritis.
Australian J. Exp. Biol. & Med. Sc., 34: 143, 1956.

132. Riedel, (1874).
Quoted by Herring (66).

133. Rinehart, J.F.
Fine structure of the renal glomerulus as revealed by electron microscopy.
Arch. Path., 59: 439, 1955.

134. Rinehart, J.F., Farquhar, M.G., Jung, H.C. and Abul-Haj, S.K.
The normal glomerulus and its basic reactions in disease.
Am. J. Path., 29: 21, 1953.

135. Robb-Smith, A.H.T.
The reticulin riddle.
J. Mt. Sinai Hosp., 24: 1155, 1957.

136. Robertson, D.M. and More, R.H.
Structure of glomerular axial region in normal and nephritic rabbits.
Arch. Path., 72: 331, 1961.

137. Ruyter, J.H.C.
Ueber einen merkwürdigen Abschnittder Vasa afferentia in dei Mauseniere.
Z. Zellforsch., 2: 242, 1925.

138. Sabour, M.S.
Diabetic Nephropathy.
Brit. J. Clin. Practice, 14: 667, 1960.

139. Sabour, M.S., El-Mahallawy, M.S. and Abou-El-Naga, I.
The renal complications of diabetes mellitus: Needle biopsy study of the renal histopathology.
Scott. Med. J., 6: 495, 1961.

140. Sakaguchi, H.
Fine structure of the renal glomerulus.
Keio. J. Med., 4: 103, 1955.

141. Schumlansky, A.
Anatomicam de renum structura.
Argentorali, 1782. Quoted by Bowman, 1842.

142. Semple, S.J.G. and de Wardenei, H.E.
Effect of increased renal venous pressure on circulatory "autoregulation" of isolated dog kidneys.
Circul. Res., 7: 643, 1959.

143. Siadat-Pour, A.
Die Ultrastruktur des Blut-Harnweges inverschiedenen Abschnitten des Nephrons bei der Maus.
Beitr. path. Anat., 120: 382, 1959.

144. Sitte, H.
Veränderungen im Glomerulum der Rattenniere nach Fremdeiweiss gaben und hypothetische Erklärung der glomerulären Ultrafiltration.
Verhandl. deutsch. Ges. Path., 43: 225, 1959.

145. Sjostrand, F.S.
Uber die Eigenfluoresenz trierischer Gewebe mit besonderer Berücksichtingung der Saugetierniere.
Acta Anat., (Suppl. I), p. 177, 1944.

146. Sjostrand, F.S. and Rhodin, J.
The ultrastructure of the proximal convoluted tubules of the mouse kidney as revealed by high resolution electron microscopy.
Exper. Cell Research, 4: 426, 1953.

147. Smith, H.W.
The Kidney: Structure and Function in Health and Disease.
Oxford University Press, New York, 1951.

148. Spargo, B., Straus, F. and Fitch, F.
Zonal renal papillary droplet change with potassium depletion.
Arch. Path., 70: 599, 1960.

149. Spiro, D.
The structural basis of proteinuria in man.
Am. J. Path., 35: 47, 1959.

150. Suzuki, Y.
An electron microscopy of the renal differentiation II. Glomerulus.
Keio J. Med., 8: 129, 1959.

151. Thayssen, W.
Centr. Med., 11: 594, 1873.

152. Thin Sectioning and associated technics for electron microscopy.
1959 - Servall-Norwalk, Connecticut, U.S.A.

153. Thoenes, W.
Die Mikromorphologie des Nephron in ihrer Beziehung zur Funktion.
Teil I: Funktionseinheit: Glomerulum- Proximales und distale Konvolut.
Klin. Wschr., 39: 504, 1961.

154. Tobian, L.
Physiology of the juxtaglomerular cells.
Ann. Int. Med., 52: 395, 1960.

155. Tobian, L., Romboulian, A. and Janecek, J.
The effect of high perfusion pressures on the granulation of juxtaglomerular cells in an isolated kidney.
J. Clin. Investigations, 38: 605, 1959.

156. Toldt, R.
Untersuchungen über das wachstum der mieren das menschen und der saugethiere.
Sitzber. Akad. Wiss Wien Math. naturw. Kl., 69: 123, 1874.

157. Ultramicrotome: A.F. Huxley Pattern. Cambridge.

158. Vimtrup, B.
On the number, shape, structure and surface area of the glomeruli in the kidneys of man and mammals.
Am. J. Anat., 41: 123, 1928.

159. Wallenius, G.
Renal clearance of dextran as a measure of glomerular permeability.
Acta Soc. Med. Upsaliensis, 1954, (Suppl. 4) p. 1-91.

160. Watson, M.L.
The use of carbon films to support tissue sections for electron microscopy.
J. Biophysic. and Biochem. Cytol., 1: 183, 1955.

161. Watson, M.L.
Carbon films and specimen stability.
J. Biophysic. and Biochem. Cytol., 2: No. 4, Suppl. 31, 1956.

162. Wiedman, M.L., Dunihue, F.W. and Robertson, W.V.B.
Effect of sodium chloride intake and mineralo-corticoid level on the granularity of juxtaglomerular cells.
J. Endocrinol., 17: 261, 1958.

163. Wirz, H.
The location of antidiuretic action in the mammalian kidney, in The Neurohypophysis, p. 157.
Editor: H. Heller, Academic Press Inc., London, 1957.

164. Yamada, E.
The fine structure of the renal glomerulus of the mouse.
J. Biophysic. & Biochem. Cytol., 1: 551, 1955.

165. Zimmermann, K.W.
Uber den Ban des Glomerulus der Saugerniere Weitere Mitteilungen.
Ztschr. mikroscop-anat. Forsch., 32: 176, 1933.

THE MORPHOGENESIS AND PATHOGENESIS OF THE DIABETIC RENAL LESIONS.

THE MORPHOGENESIS AND PATHOGENESIS OF THE DIABETIC RENAL LESIONS.

Introduction.

The renal complications of diabetes mellitus attracted sufficient attention only in 1936, when Paul Kimmelstiel and Clifford Wilson (33), during their study on the pathology of the kidney in various diseases, noted a lesion which had not been previously described. These two pioneer authors described hyaline eosinophilic nodules in the centre of the glomeruli suggestive of amyloid disease, but giving negative reactions to amyloid stains, and called it "intercapillary glomerulosclerosis". They considered the lesion to represent a broadening of the intercapillary connective tissue.

In Japan, at the same time (1936), Murakami (40), described these nodular hyaline lesions in the glomeruli of diabetics, but his work did not receive so much attention as the paper of Kimmelstiel and Wilson mentioned above.

The morphogenesis of the hyaline glomerular nodules as described by Kimmelstiel and Wilson was criticised by Allen (2). He considered that it is <u>not</u> an intercapillary mass of hyaline which extends from the hilar arterioles to the periphery of the glomerulus, as Kimmelstiel and Wilson had believed, but rather a mass of hyaline formed by abrupt thickening of the wall of one or more closely situated capillaries. This intramural histogenesis of the nodular lesions has also been stressed by Bell (11), and by Sabour, El-Mahallawy and Abou-El-Naga (44), although McManus (37) agrees with the original belief of Kimmelstiel and Wilson regarding the intercapillary origin of these nodules. Plugging of the glomerular capillaries

by protein coagulum deposited from the blood was suggested by Anderson in 1954 (3) and has constituted a third view for the morphogenesis of the Kimmelstiel-Wilson (K.W.) hyaline nodules.

In 1943 and 1944, Spühler and Zollinger (45,46) described in considerable detail another glomerular lesion found predominantly in the kidneys of diabetics with severe renal damage, and called it a "glomerular fibrinoid cap". These papers were followed by one by Wernly in 1944 (47), who in a series of ingenious diagrams illustrated the relationship of nodular lesions and hyaline glomerular caps, and considered the two as being distinctly different. Later, Hall (29) briefly described what he termed "the exudative lesion of diabetic glomeruli,"; Barrie, Askanazy and Smith (6) described the presence of "fibrin caps" in glomeruli of diabetics; and Koss (34) and Sabour et al (44), described the same lesion in great detail and called it "hyaline fibrinoid lesion of diabetes."

A third type of glomerular lesion in the kidneys of diabetics was described by Bell in 1946 (11). This, he called "diffuse glomerulosclerosis". It consisted of a diffuse thickening of the capillary walls and he considered it as characteristic of diabetes as the nodular lesion.

A fourth glomerular lesion has also been described. Allen (2) in 1941 described capillary micro-aneurysms in the glomeruli and considered them important in the production of albuminuria. He suggested that they resulted from obstruction to the capillary outflow by the K.W. nodules. Ashton in 1950 (4), by injection of Indian ink, beautifully demonstrated these glomerular aneurysms and drew attention to the similarity in the changes of diabetic retinopathy to those of diabetic glomerulosclerosis.

Anderson (3), who had found glomerular aneurysms in cases which showed no nodular lesions, considered that the K.W. nodules developed by plugging of these capillary micro-aneurysms with a protein coagulum from the blood.

The studies referred to above were obtained from necropsy material. They described therefore, advanced renal changes, the morphogenesis and pathogenesis of which could not be easily ascertained. In a detailed study reported by Sabour and co-workers in 1961 (44) renal tissue from 75 diabetic patients was obtained by needle biopsy of the kidney and a description of the evolution of the diabetic lesions was attempted. Dilatation of the glomerular capillaries, sometimes reaching micro-aneurysmal proportions and diffuse thickening of the glomerular capillary walls were found to be early diabetic changes, while the K.W. nodules were rather late in development and the fibrinoid lesions were only found in advanced cases with a great degree of renal damage. On their morphogenesis, those authors believed that diffuse and nodular glomerulosclerosis were lesions in the glomerular capillary basement membrane and were neither "intercapillary" nor "intracapillary": while they considered the fibrinoid lesions to be intraluminal. They suggested that diffuse and nodular glomerulosclerosis are the result of excessive deposition of mucopolysaccharides from the blood, while the fibrinoid lesions were lipoprotein in nature and are similarly deposited in the glomeruli from the serum.

The pathogenesis of the diabetic renal lesions was extensively studied in the past decade. Various metabolic and endocrine disturbances have been described in association with the specific vascular lesions of diabetes - namely retinopathy and nephropathy. It is claimed that many of these

disturbances have a pathogenic relationship with the development of the vascular complications.

Becker (7) in 1952 gave evidence of excessive adrenal cortical function in diabetics with retinopathy and nephropathy. He noted an apparent relationship between the lipoid-laden vacuolated cells in the zona fasciculata of the adrenal cortex and the K.W. lesions in the kidney. A picture similar to early diabetic retinopathy and the K.W. lesion could be produced in alloxan-diabetic rabbits by the injection of corticotrophin or cortisone. Becker, Allen et al (8), Becker, Maengwyn-Davies et al (10) gave further evidence of excessive production of adrenal glucocorticoids in patients with diabetic retinopathy.

Becker et al (9) in 1953, also reported a disturbance of vitamin B_{12} metabolism in patients with diabetic retinopathy. Using the vitamin B_{12} excretion test, they found that diabetics with retinopathy cannot retain the vitamin, while those with no retinopathy retain most of the given dose. On the other hand, Field and co-workers in 1957 (25), could not find a definite relationship between the development of degenerative complications of diabetes and the excretion patterns of the B vitamins.

A disturbed carbohydrate metabolism has been found in patients with clinically evident diabetic nephropathy. Thus, Berkman et al (13,14) in a study of the serum polysaccharides in diabetics and non diabetics, reported an increase in the total serum polysaccharides in diabetics with degenerative vascular complications, the highest level being observed in those with clinically evident nephropathy (1). It is not clear whether this increase is due to the renal impairment and is thus the result of the diabetic nephropathy, or whether it precedes the nephropathy and provokes

the pathological lesions in the kidneys.

Main et al in 1949 (39), have noted hypercholesterolaemia in association with diabetic glomerulosclerosis. Later, Barr and Russ (5), Engelberg et al (22,23), Keiding et al (32) and Collens et al (17) studied the serum lipids, lipoproteins and cholesterol levels in diabetics and their relationship to degenerative vascular complications, and found that the serum cholesterol and phospholipid levels were usually raised. The most striking finding, however, was an increase of the S_f 12-20 class of lipoproteins in the serum of patients with diabetic nephropathy out of proportion to the raised level of cholesterol.

In an exhaustive study of the endocrine and metabolic aspects of diabetic nephropathy El-Mahallawy, Sabour, Osman and Sadek (21) in 1960 have arrived at the conclusion that a multifacetted, metabolic upset is present in the diabetic patients and is responsible for the development of the renal lesions: a disturbance in protein metabolism resulting in a rise in α 2 and β-globulin; a disturbance in lipid metabolism causing lipaemia and especially a rise in the smaller aggregates of serum lipoproteins; a disturbance in carbohydrate metabolism leading to a rise in the serum polysaccharides; excessive glucocorticoid activity; and inability to retain vitamin B_{12}. These authors carried out their biochemical and endocrine function studies in the same patients from whom renal tissue was obtained and examined pathologically and histochemically. Therefore, they could hypothesise that the different renal lesions are related to different metabolic and endocrine disturbances. They maintained that the high serum mucopolysaccharides were probably related to the development of the lesions of diffuse and nodular glomerulosclerosis; while the disturbed

lipoprotein metabolism, vitamin B_{12} metabolism, and the excessive glucocortical activity were probably related to the development of the hyaline fibrinoid lesions.

Recognition of the degenerative lesions which develop in patients with long-standing diabetes had led to a controversy as to whether these are part of the natural history of the disease or complications of it. The former view is held by Dolger (18), Larsson and his co-workers (35), and McNeal and Rogers (38) while many workers such as Wilson, Root and Marble (50), Dunlop (20), Hardin and his associates (30) and White (48) regard the lesions as complications. One of the main points of issue is whether good control of the diabetic state over the years protects the patient from the degenerative changes: if they are an integral part of the disease they will probably develop in time irrespective of treatment. Dolger (19) has reported that these specific degenerative lesions may even appear, in the elderly group of patients, before hyperglycaemia and glycosuria become clinically evident.

An electron microscopic study was thought valuable to unravel some of the still controversial points about the morphogenesis and the pathogenesis of the diabetic renal lesions and to show whether they are an integral part of the disease or complications. This study was designed to include two aspects of the condition.

First: An electron microscopic examination of renal biopsy material from young diabetic patients with recent onset of diabetes.

Second: An electron microscopic examination of the kidneys of rabbits given prednisolone until they developed glycosuria and proteinuria.

The aim of the first part of the study was to see whether such

clinically noncomplicated diabetic patients show a lesion in their kidneys coincident with the recognition of the metabolic defect or have normal renal ultrastructure at this stage. The aim of the second part of the study was to demonstrate exactly which of the various types of diabetic renal lesions will result from administration of an excess of the glucocorticoid hormone.

I. Electron Microscopy of Diabetic Glomeruli.

This investigation consists of the electron microscopic examination of renal biopsies from young patients suffering from diabetes, whose disease had been diagnosed for periods varying from 5 weeks to 5 years. None of these patients showed any clinical or laboratory evidence of nephropathy. Electron microscopic studies have been reported from patients showing such clinical evidence (12,24) and chracteristic changes have been described. Goetz, Hartman and Lasarow have briefly reported electron microscopic changes in the glomeruli of patients within one or two years of the onset of diabetes in the absence of a clinically evident renal lesion (28) but these authors published no microphotographs to illustrate their findings.

Material.

Four diabetic patients were shosen for the study: the time elapsing between the diagnosis of the disease and the renal biopsy varied from 5 weeks to 5 years. The patients had varying degrees of severity of diabetes as judged by the fasting blood sugar level and the units of insulin necessary to control the glycosuria. In none of the patients studied was there any clinical evidence of renal involvement; they had no proteinuria, no oedema, normal blood pressure and the creatinine clearance was

normal in all. In addition, two other patients were studied. The first was an elderly patient with a fairly recent onset of diabetes, in order to see whether there is any difference between the juvenile and the maturity onset types of diabetes. The second patient was a juvenile diabetic who had her diabetes for 12 years and who began to show clinical evidence of renal involvement; she had mild proteinuria and slightly impaired creatinine clearance. This patient was studied in order to try to follow the evolution of the lesions which have been found in the kidney of the early diabetic patients. The pertinent clinical and laboratory data are shown in Table 4.

Percutaneous needle biopsy of the kidney was performed, using a Franklin-Silverman needle, and the core of renal tissue obtained was divided into two parts: one was fixed in corrosive formol for light microscopic examination and the other was fixed in 1% buffered osmium tetroxide, embedded in methacrylate (as mentioned before) and examined by the electron microscope.

Results.

On light micro s copy, all the patients showed normal renal glomerular histology.

On electron microscopy, the diabetic glomeruli showed thickening of the capillary basement membrane (Fig. 76 & 77). This was a constant finding seen in all the glomeruli examined in all the patients, although the degree of thickening varied from one glomerulus to another and from one loop to another in the same glomerulus (Fig. 78). The average thickness of the basement membrane in the five patients with a recent onset of

TABLE 4.

Clinical and Laboratory Data of Diabetic Patients
Studied by Electron Microscopy.

Patient	Sex	Age Years	Duration of Diabetic Symptoms.	Daily Insulin Requirement Units.	Protein-uria	Oedema	B.P.	Retina	Blood urea nitrogen mg/100 ml.	Creatinine Clearance Ml/min.
T.D.	M	32	5 weeks	30	-	-	130/80	Normal	13	91
R.S.	M	25	2 months	36	-	-	120/80	Normal	12	120
M.Mc.	M	26	2 years	56	-	-	130/80	Normal	18	112
T.C.	M	37	5 years	44	-	-	120/70	Normal	17	115
R.M.	M	66	4 years	Tolbutamide	-	-	170/100	Bilateral cataract	22	100
J.B.	F	24	12 years	60	+	-	160/90	Micro-aneurysms	29	60

diabetes was 4000-6000 Å , i.e. two to three times the normal thickness. No abnormality was seen in the fine structure of the basement membrane even when examined at very high magnification (Fig. 79), no gaps or local degenerative changes, as are sometimes observed in the basement membrane of patients with membranous glomerulonephritis, could be demonstrated.

In several places, masses of basement membrane-like material appeared to be intermingled with the endothelial cytoplasm or in some cases probably deposited in the cytoplasm of an endothelial cell (Fig. 80). This was particularly obvious within the "axial" endothelial cells (Fig. 81). However, in the region of these cells, the basement membrane tended to be rather tortuous, and the appearance of deposition of basement membrane material in the cells may be due, simply to plane of section. This is not sufficient to explain all the basement membrane material seen within the endothelial cytoplasm because it has been repeatedly shown that "spurs" of basement membrane do exist (Fig. 80 & 81). This "growth" of spurs of basement membrane material within the endothelial cytoplasm can easily explain the known finding by light microscopy that in diabetic glomerulosclerosis the capillary wall looks thick and fibrillar.

The attenuated part of the endothelial cytoplasm showed increased thickness in many areas (Fig. 82 & 83). This, together with the thick basement membrane, encroached on the capillary lumen, and in some areas actually occluded the lumen (Fig. 81 & 84).

The epithelial cells in general, showed no change: the pedicels and the slit pores were normal in appearance and size. Small hyaline bodies were seen within some epithelial cells (Fig. 82 & 85). They were quite

different in appearance from mitochondria even when small, and no intermediate or transitional forms from mitochondria were observed. Similar hyaline bodies, but usually larger in size, were occasionally seen in the cells of the proximal convoluted tubules. (Fig. 86). These hyaline bodies are the "protein-absorption droplets" of light microscopists (41) and may be an early electron microscopic evidence of excess protein loss through the glomerular filter, the protein being taken up by micropinocytosis by the glomerular epithelial cells, and from the tubular fluid by the cells of theproximal convoluted tubules. This finding is not specific for diabetes; similar hyaline droplets have been found in patients suffering from various conditions producing the nephrotic syndrome and have been observed in patients with postural proteinuria.

The basement membrane of Bowman's capsule was approximately 10 times thicker in the diabetic than in normal glomeruli: it measured more than 1.5 μ in thickness in all the glomeruli examined (Fig. 87). Occasionally in addition to its diffuse thickening, it bulged into a large nodular mass of basement membrane material (Fig. 88). Some of the lining cells were abnormal; their cytoplasm was strongly osmiophilic and was vacuolated, the vacuoles lying in a subnuclear position, displacing the nucleus towards Bowman's space (Fig. 89,90).

No exudative "fibrinoid" deposits were detected in the glomeruli of any one of these patients.

The endothelial lining cells of some glomerular arterioles from these diabetic patients were swollen, vacuolated and strongly osmiophilic (Fig. 91). No hyaline or fibrinoid deposits were seen in the glomerular arterioles.

Marked thickening of the basement membrane of the proximal and distal

convoluted tubules was observed (Fig. 92). An occasional necrotic, densely osmiophilic, shrunken cell, showing complete destruction of all the details of the intracellular structure and contents, was observed in the collecting tubules.

Exactly the same changes as described above, and in the same degree were seen in the glomeruli of patient R.M., the elderly man who had his diabetes diagnosed 4 years prior to the renal biopsy.

Patient J.B., the diabetic for 12 years, had the same lesions but were more marked in degree. The average glomerular capillary basement membrane thickness was 8000 Å. In addition to the diffusely thickened basement membrane, areas of focal thickening were noticed in some capillary loops (Fig. 93). These focal thickenings were sometimes as large as 1-3 μ in diameter (Fig. 94) and these could be the fore-runner of Kimmelstiel-Wilson nodules. The basement membrane was more tortuous and many endothelial cells showed large deposits of basement membrane material (Fig. 95).

More epithelial cells contained the hyaline protein-absorption droplets (Fig. 96). In some areas the pedicel arrangement of the epithelial cells was absent and the cytoplasm was directly ~~absent~~ applied to the external surface of the thickened basement membrane smearing it (Fig. 97) as is seen in the glomeruli of patients with membraneous glomerulonephritis. However, this was quite an uncommon finding in this patient and most epithelial cells retained the normal pedicel arrangement.

No fibrinoid lesions were noted in the glomeruli of this patient.

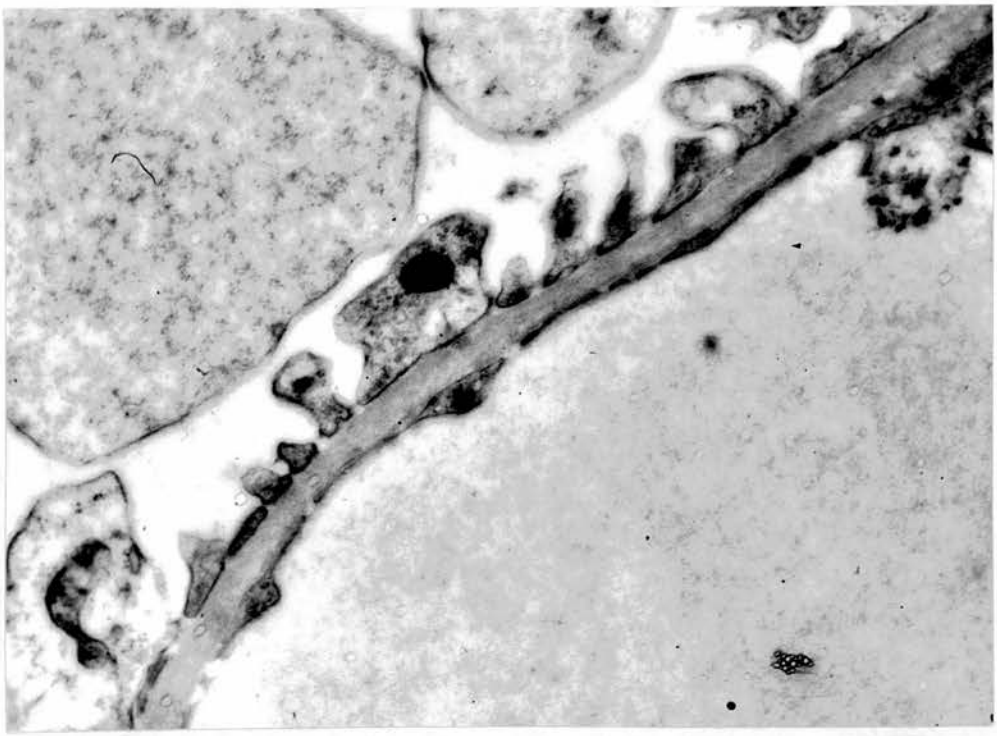


Fig. 76 Glomerular Capillary wall from a normal human subject: Note the thickness of the basement membrane. x 24,000

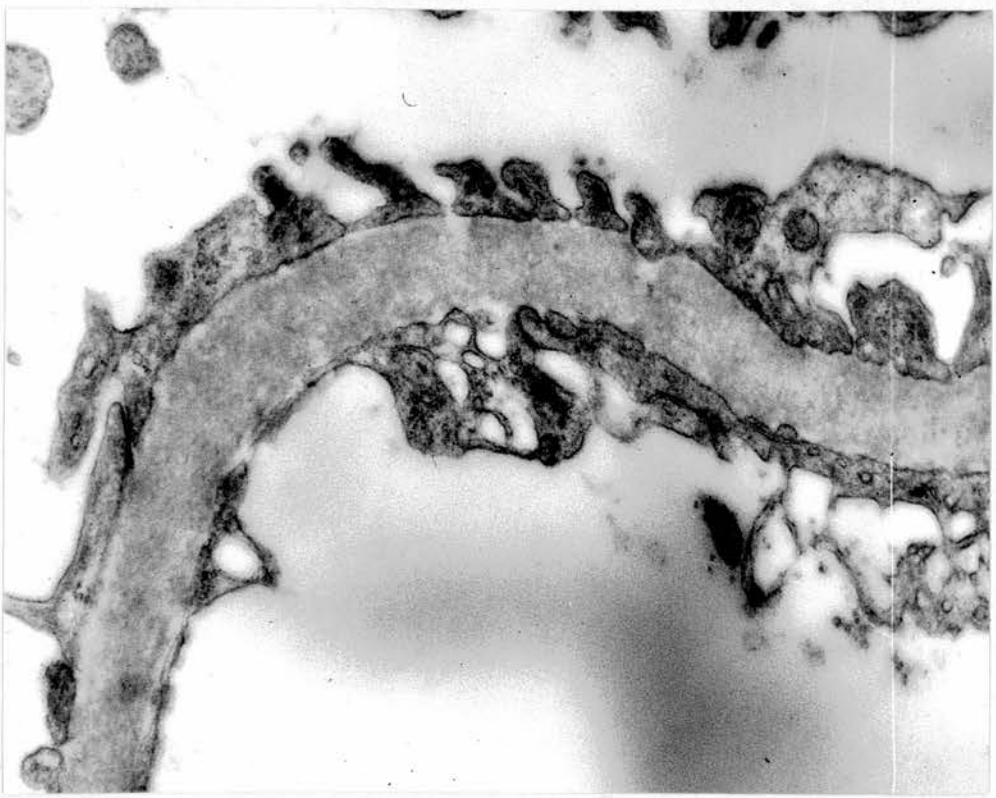


Fig. 77. Glomerular capillary wall from patient T.C., diabetic for 5 years. Note thickness of B.M. x 24,000

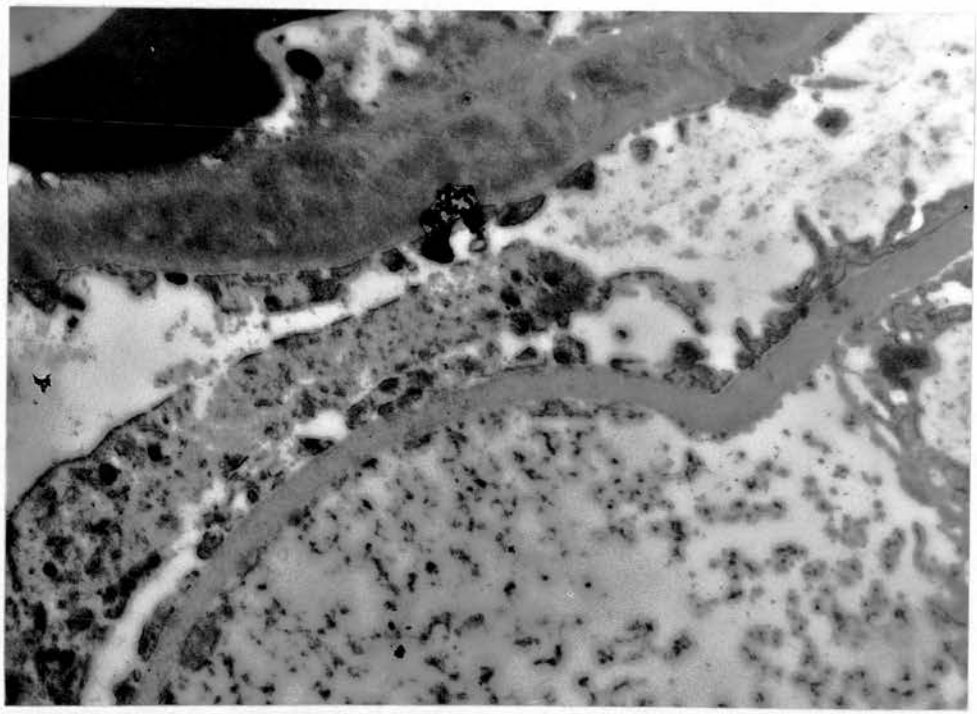


Fig. 78. Two adjacent glomerular capillaries from patient R.M. The basement membrane of the upper one is three times the normal thickness, that of the lower one is of normal thickness. x 9,000

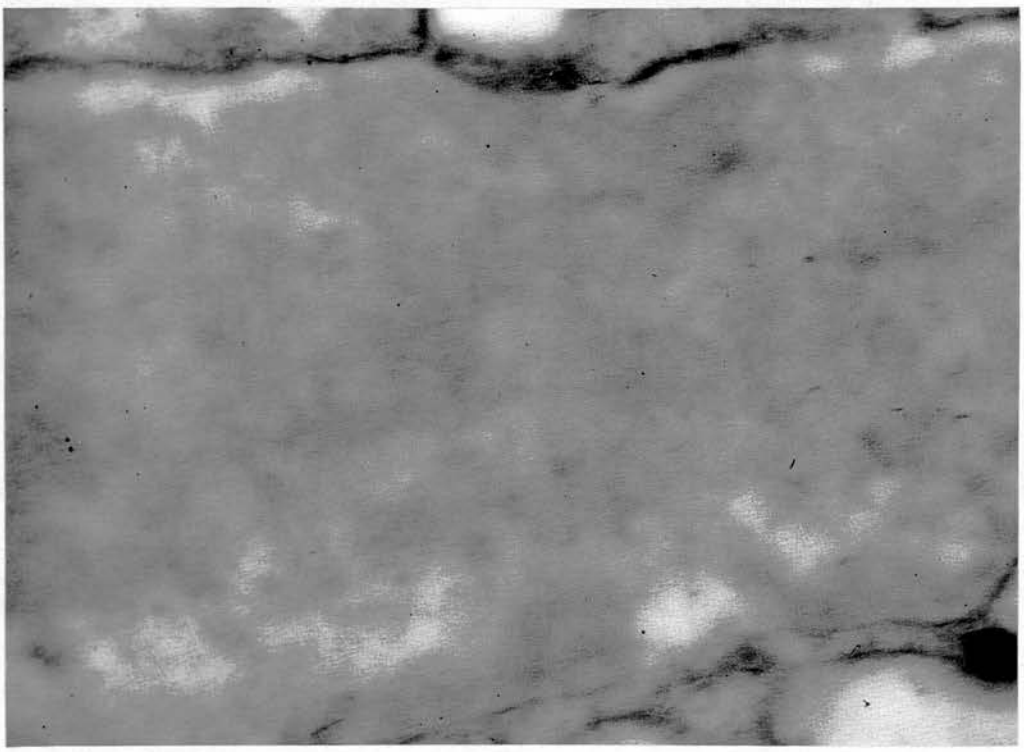


Fig. 79. Glomerular capillary wall from patient J.B. x 60,000

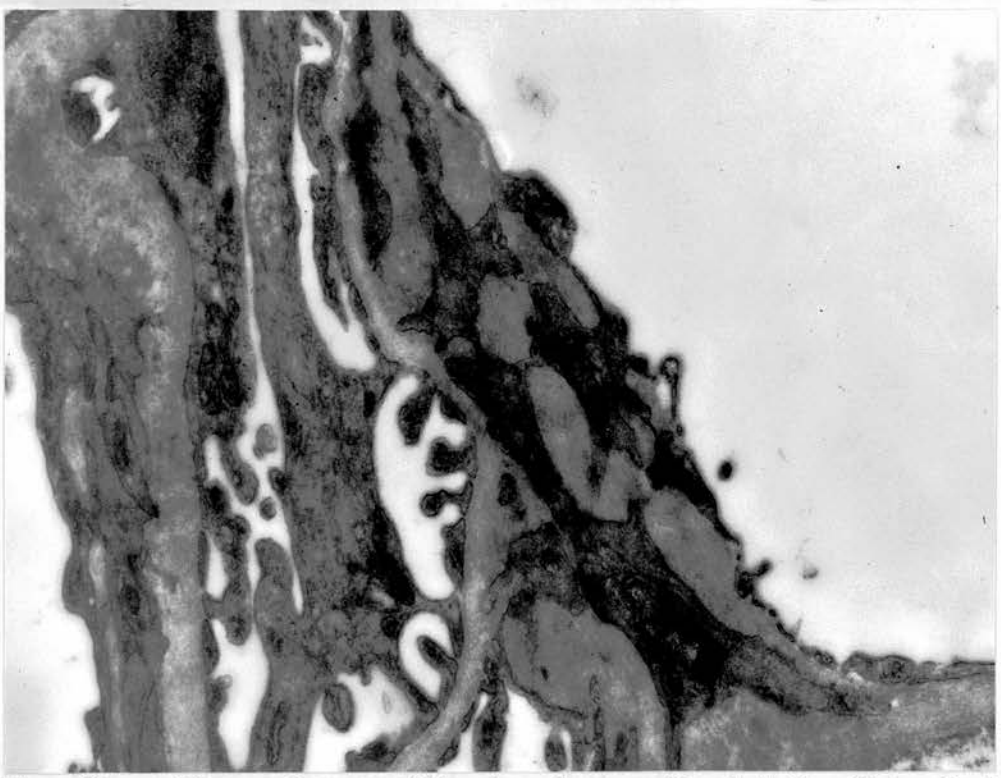


Fig. 80. Glomerular capillaries from patient T.C. Note that the basement membrane shows areas of focal nodular thickening. The endothelial cytoplasm is thickened in the capillary on the right side and "spurs" of pale basement membrane material can be seen within. x 15,000.

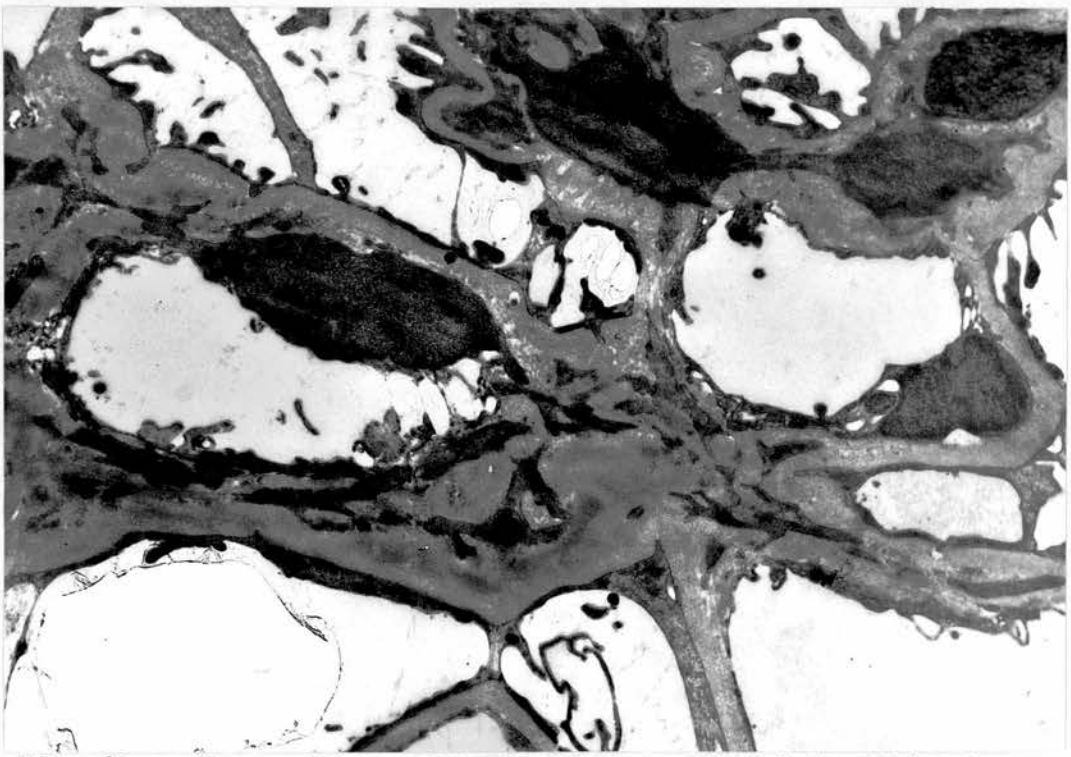


Fig. 81. Glomerular capillaries from patient T.C. Note the diffuse and focal nodular thickening of the basement membranes, the abundant endothelial cytoplasm and the encroachment on or actual occlusion of the lumina of some capillaries. x 6,000

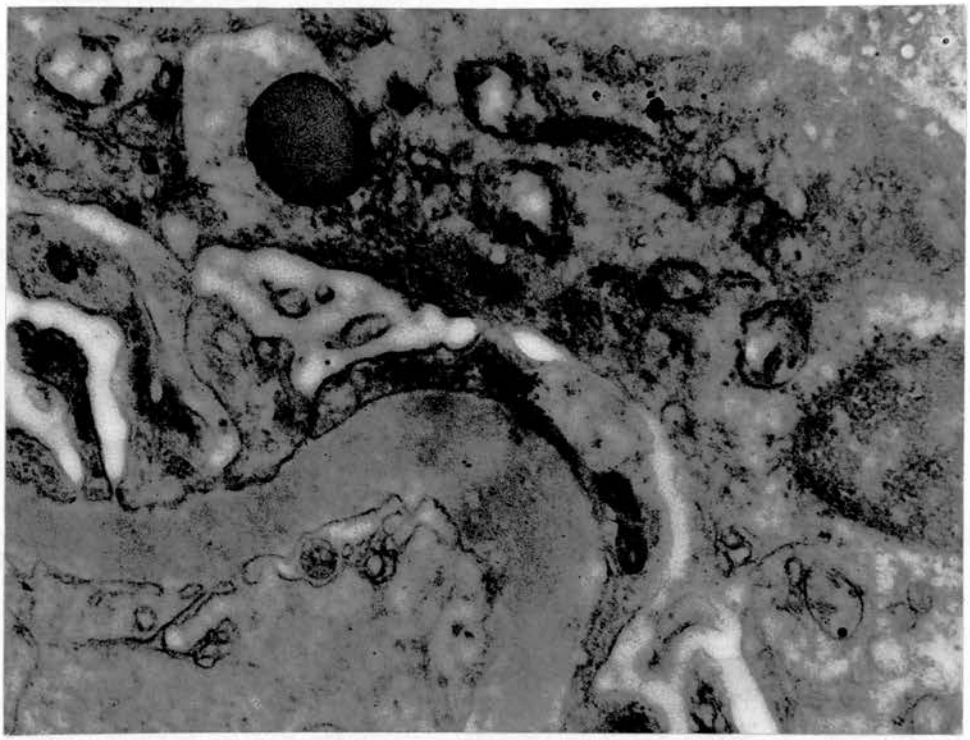


Fig. 82 Glomerular capillary wall from patient T.D., diabetic for 5 weeks. Note diffuse thickening of the capillary basement membrane and marked increase in the cytoplasm of the attenuated part of the endothelial lining. A hyaline droplet is seen in the epithelial cytoplasm. x 24,000

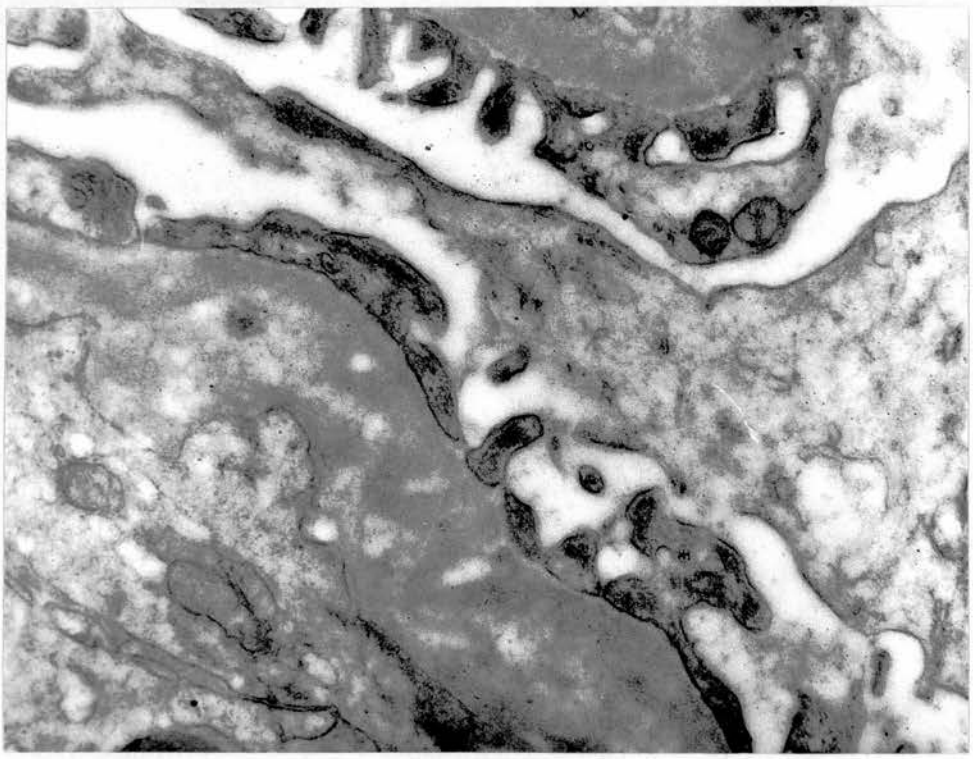


Fig. 83 Two glomerular capillary walls from patient T.D. The basement membrane and the lamina attenuata are markedly thickened. x 24,000

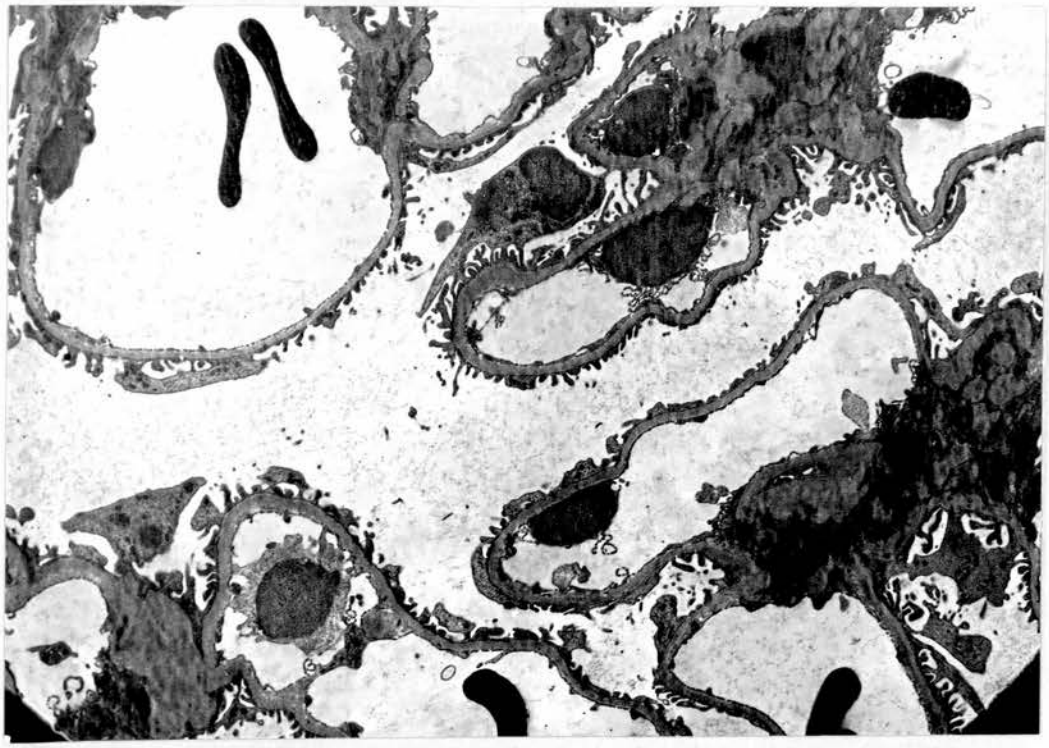


Fig. 84 Low power electron micrograph of a glomerulus from patient T.C. The thick basement membrane and endothelium and the encroachment on the capillary lumina is particularly evident in relation to the "axial" endothelial cells. x 2,500

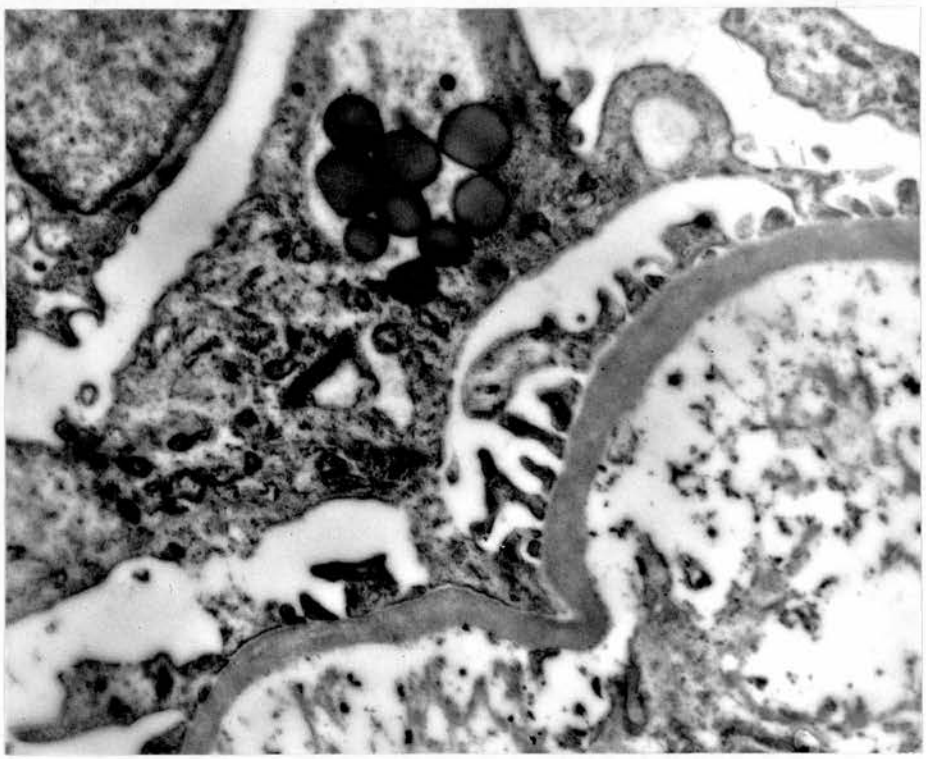


Fig. 85. Glomerular capillary wall from patient R.M. Many hyaline droplets can be seen within the epithelial cell. x 9,000

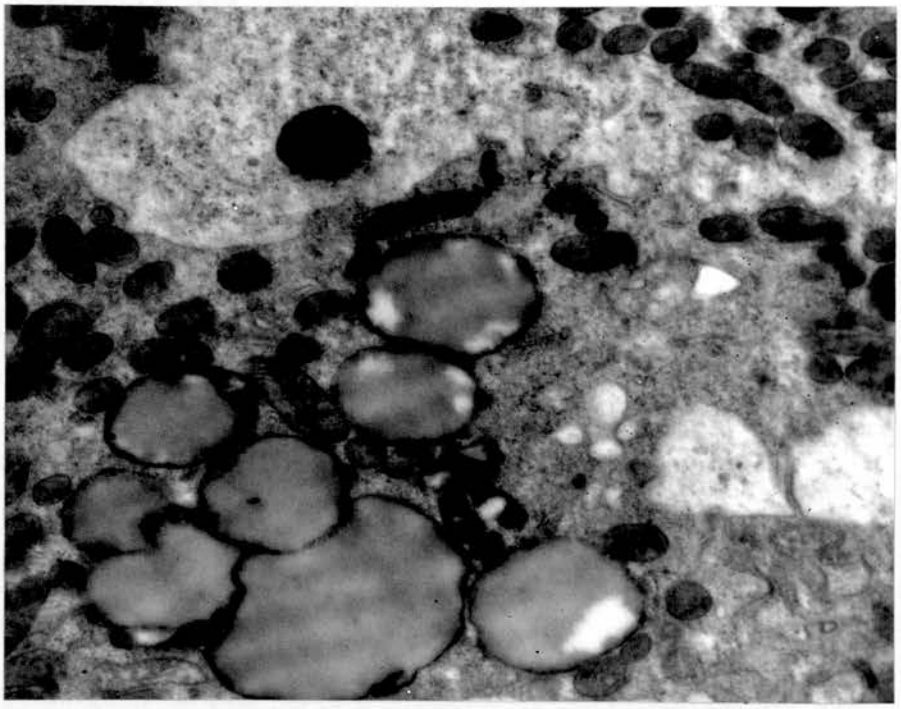


Fig. 86 Many hyaline droplets within the cytoplasm of a proximal convoluted tubule cell from patient T.C. x 20,000

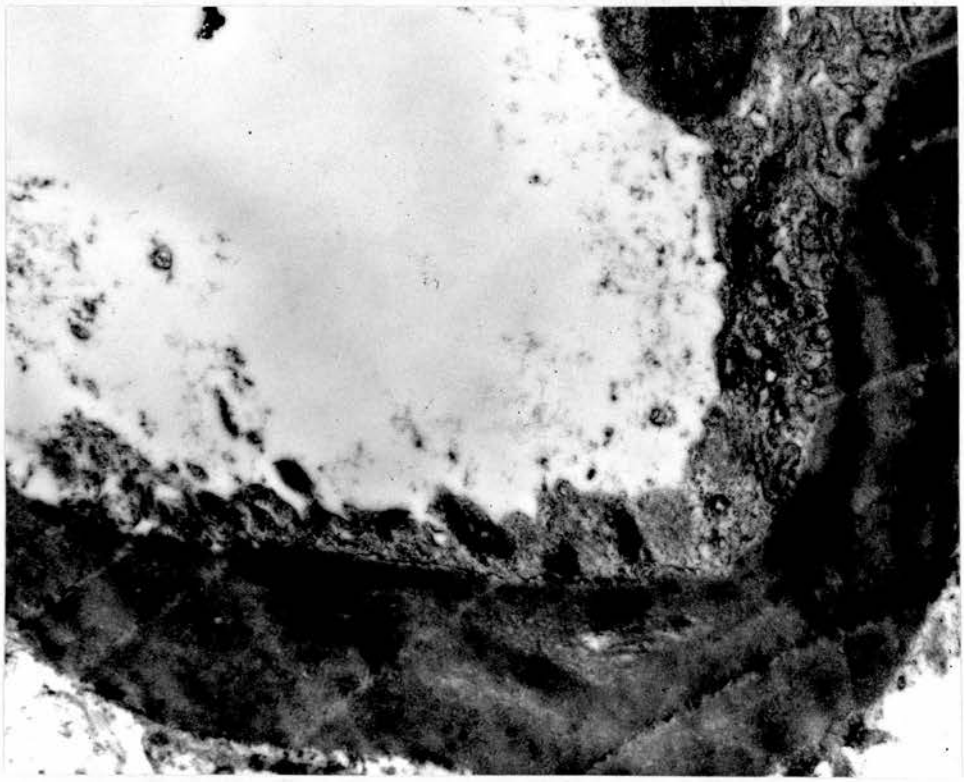


Fig. 87 Bowman's capsule from patient R.S., diabetic for 2 months markedly thickened. x 9,000

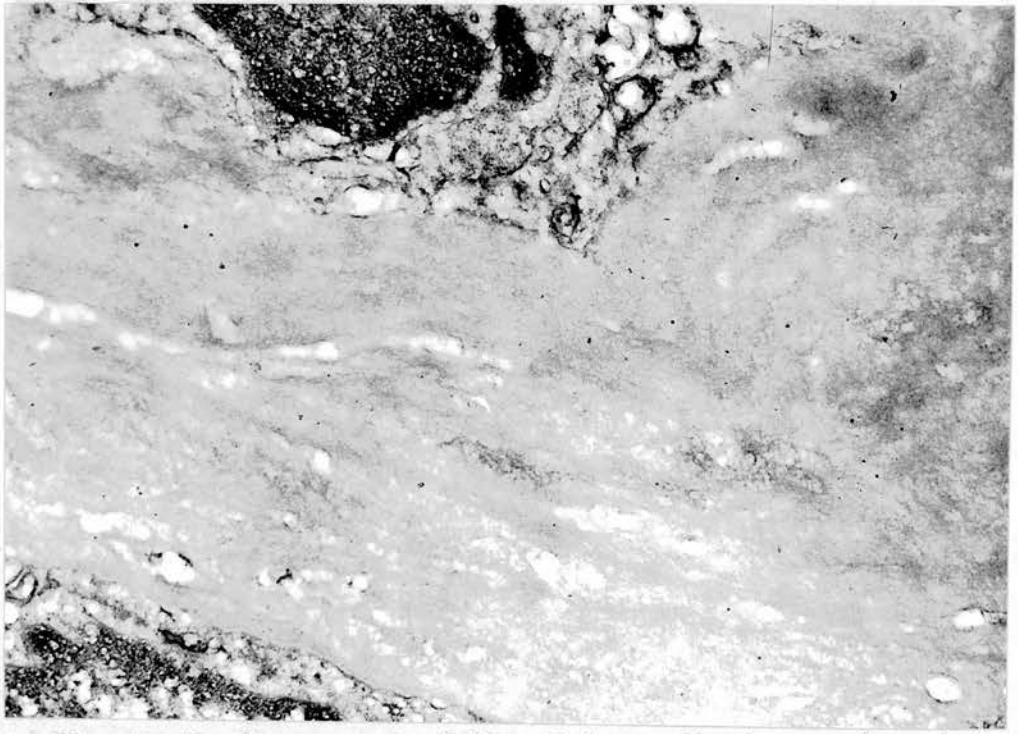


Fig. 88 In the lower part of the picture, the basement membrane of a distal convoluted tubule can be seen to be markedly thickened; just above it the basement membrane of Bowman's capsule is not only markedly thickened, but has bulged into a large nodule. From patient T.D., diabetic for 5 weeks. x 12,000

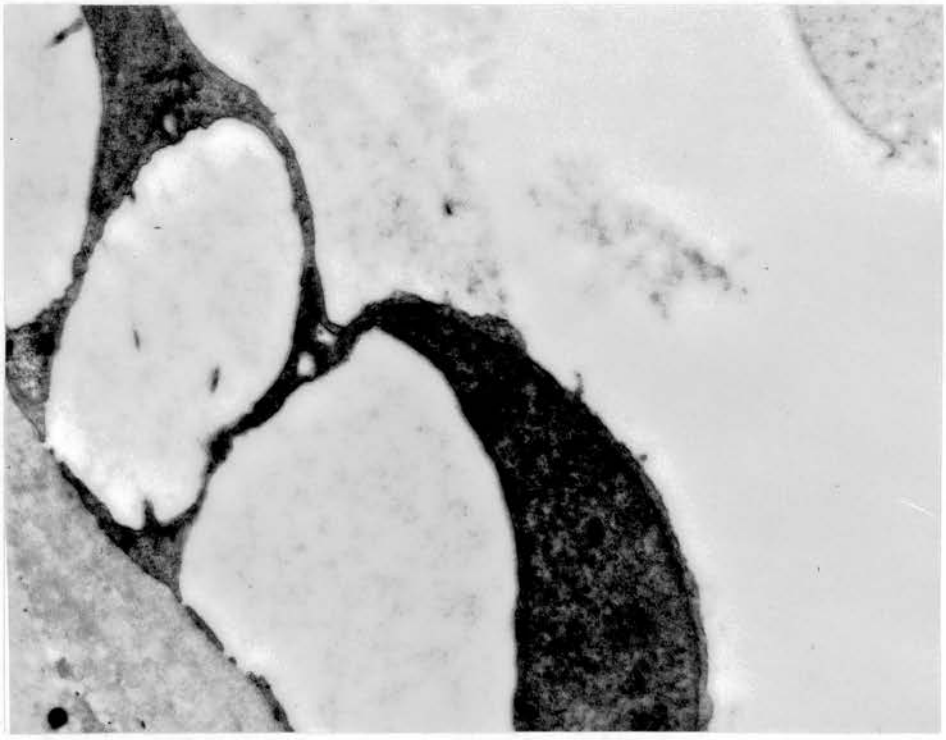


Fig. 89 B owman's capsule from patient T.C. The lining cell is swollen and vacuolated. x 12,000

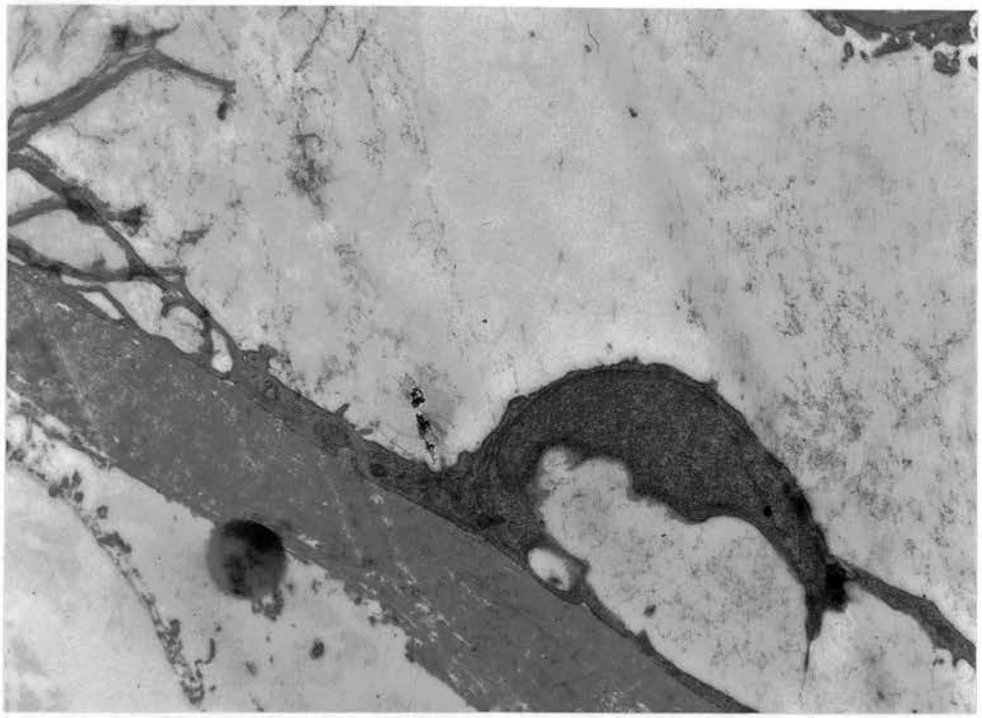


Fig. 90 Bowman's capsule from patient T.C. Note the very thick basement membrane and the swollen vacuolated lining cell. x 6,000

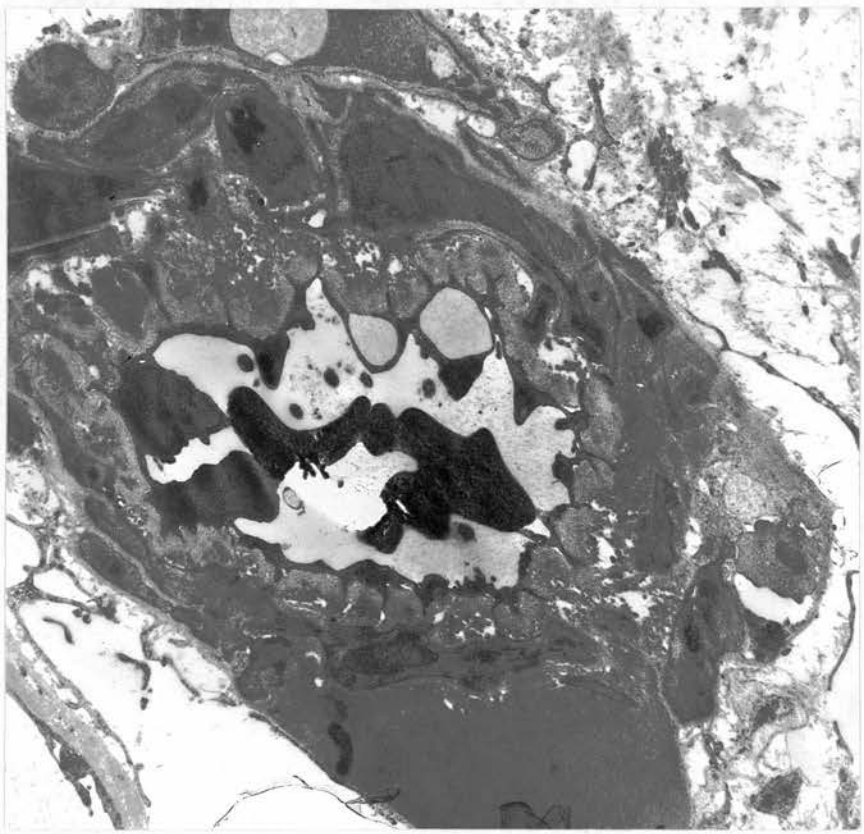


Fig. 91 Glomerular arteriole from patient T.C. The endothelium is osmiophilic and vacuolated. x 4,000

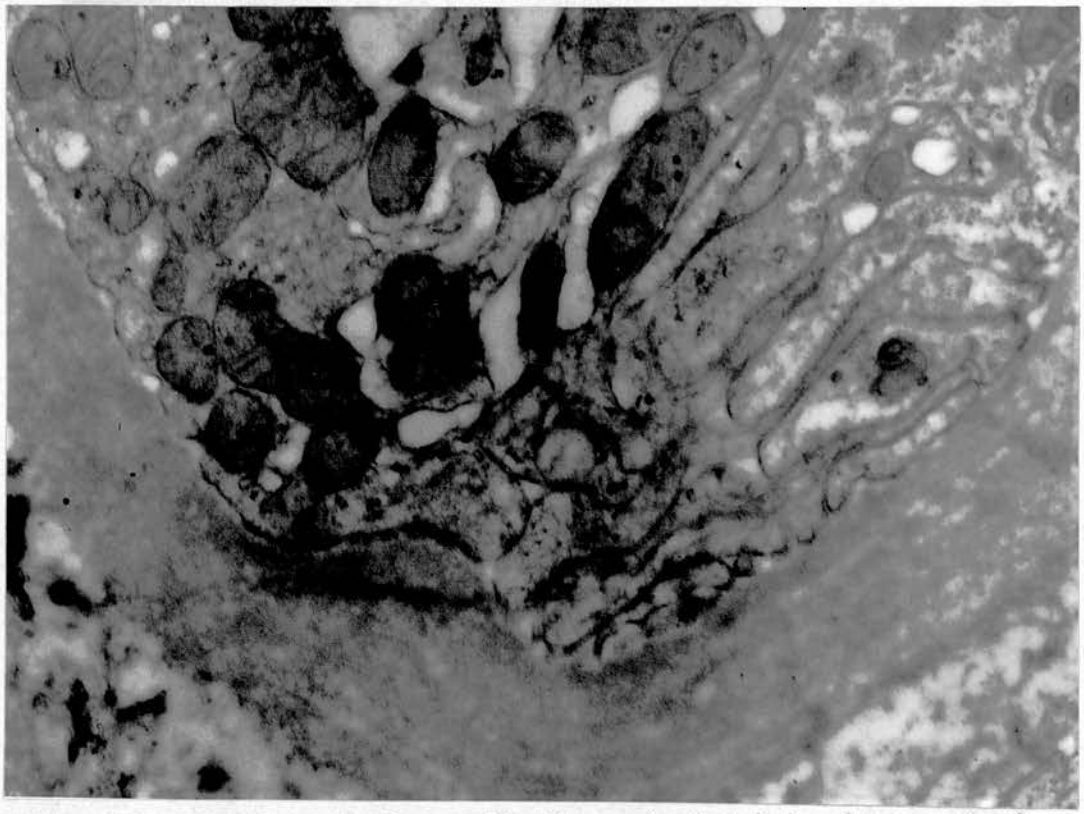


Fig. 92 Basal part of a cell of proximal tubule from patient T.D. Note marked thickening of basement membrane. x 24,000

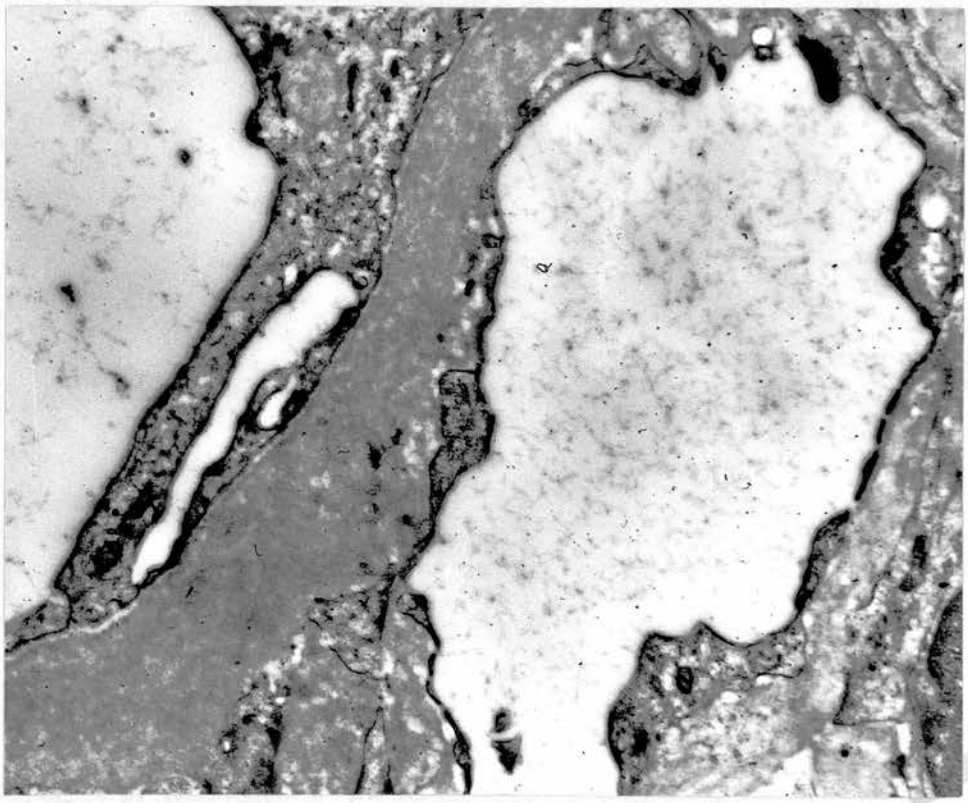


Fig. 93 Glomerular capillary wall from patient J.B., diabetic for 12 years. The basement membrane is diffusely thickened and shows additional localised focal thickening. The pedicels are lost and the epithelial cytoplasm is smeared over the outer surface of the thick basement membrane. x 12,000

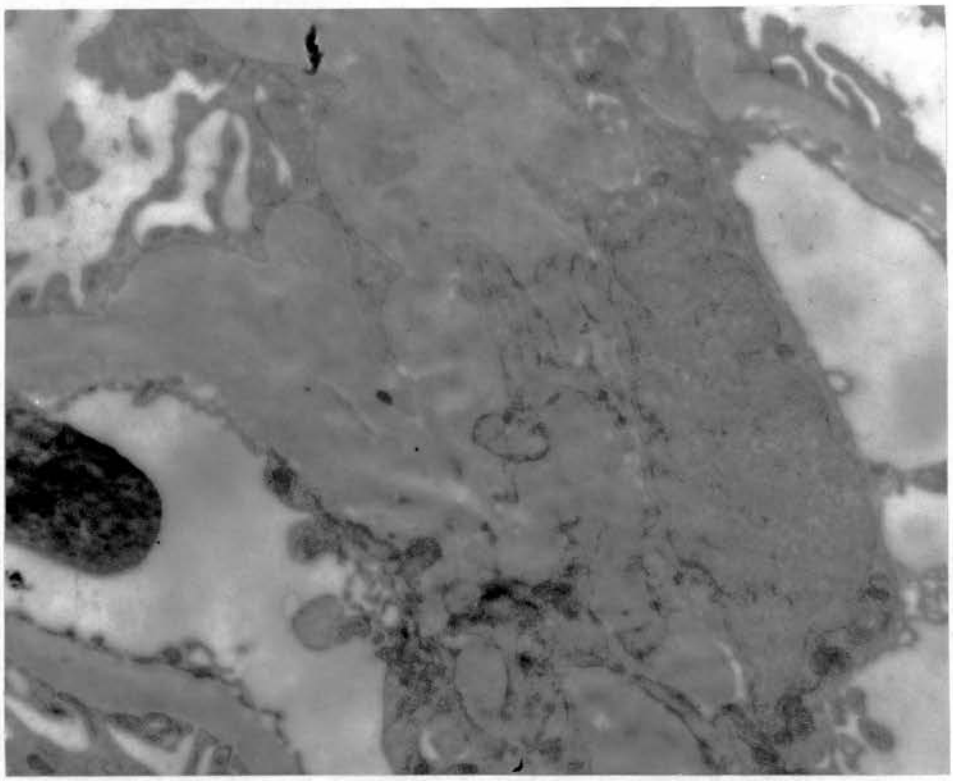


Fig. 94 Glomerular capillaries from patient J.B. Two focal thickenings opposite each other are becoming confluent, forming a larger hyaline nodule of basement membrane material. x 12,000

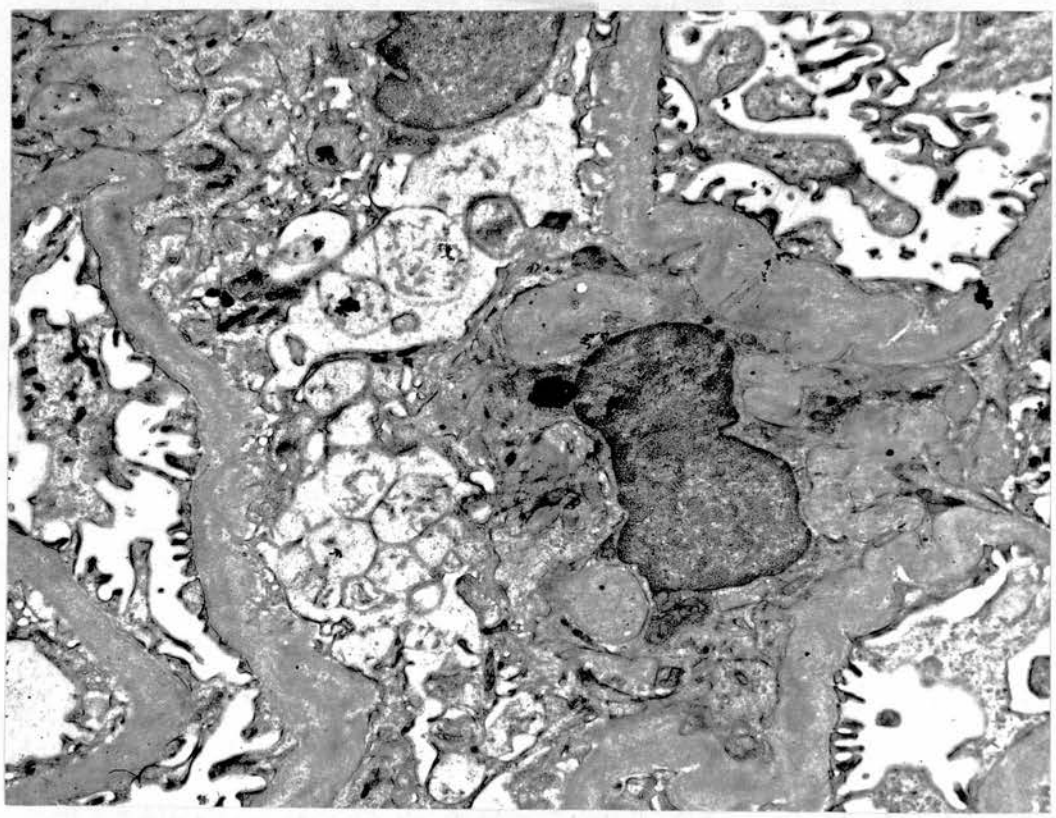


Fig. 95 Glomerular capillaries from patient J.B. Note the marked thickening and tortuosity of the basement membrane and the excess of basement membrane material within the cytoplasm of an "axial" endothelial cell. x 4,000

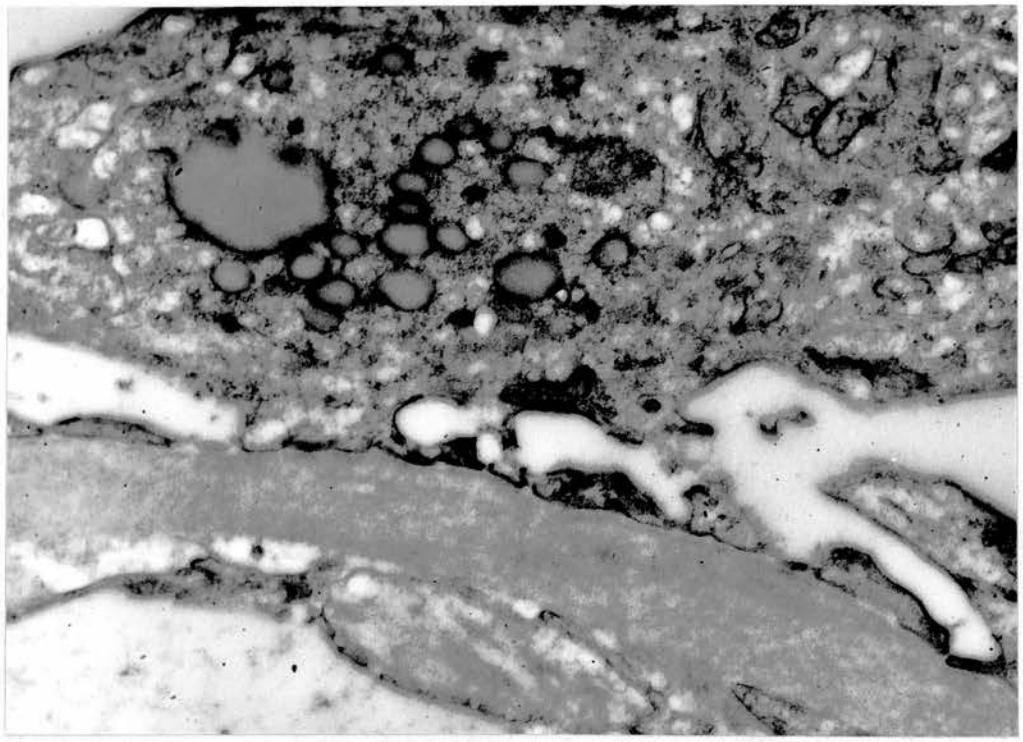


Fig. 96. Glomerular capillary wall from patient J.B. Note the thick basement membrane and the hyaline droplets in the epithelial cell. x 20,000

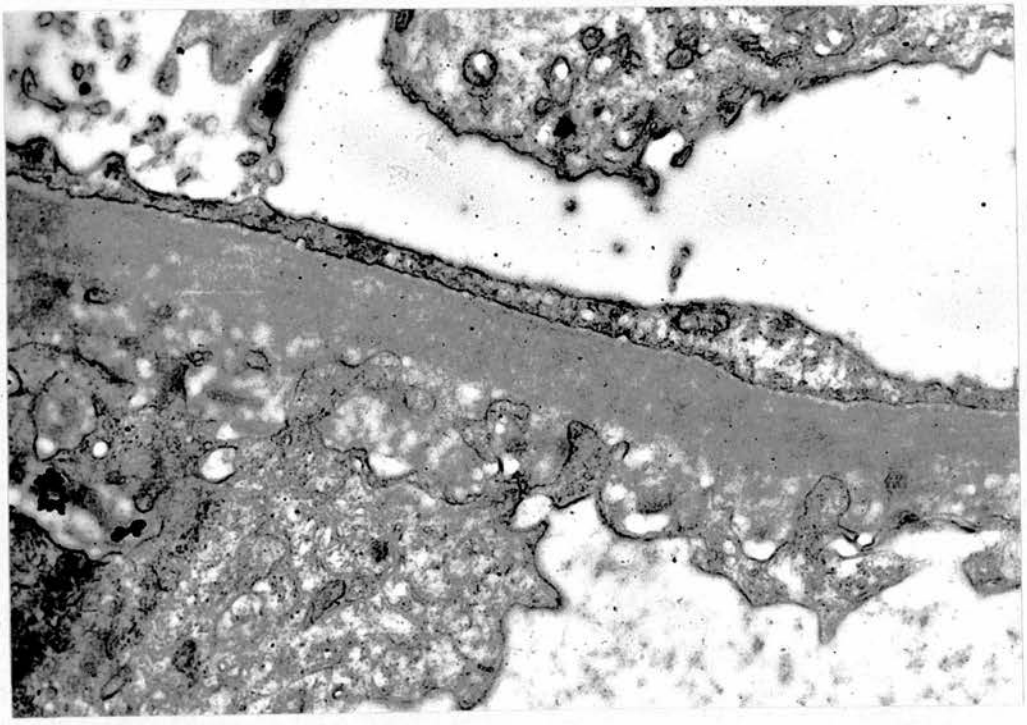


Fig. 97. Glomerular capillary wall from patient J.B. The normal pedicel arrangement is lost and the epithelial cytoplasm is spread over the outer surface of the thickened basement membrane. The lamina attenuata is thickened. x 9,000

DISCUSSION.

Thickening of the capillary basement membrane has been reported in glomeruli of diabetic patients with and without clinically evident renal involvement when examined by the electron microscope (12,24,28). In this study, thickening of the glomerular capillary basement membrane of the basement membrane of Bowman's capsule and of the tubules was found in young diabetic patients within a few weeks from the date of diagnosis of the disease. The basement membrane of the whole nephron appears therefore to be involved almost or entirely coincidentally with the onset of the clinical features of the disease.

The basement membrane is known to consist of a glycoprotein. A high level of serum polysaccharides has been reported in association with diabetic renal and retinal disease, and it has been suggested that an error in polysaccharide metabolism is responsible for the renal lesion when it develops in diabetic patients. Such abnormality may be basic and early in the syndrome of diabetes mellitus, though the serum polysaccharide levels in diabetic patients with no clinical evidence of nephropathy or retinopathy have been found to be within the normal range (1, 21).

The marked diffuse thickening of the basement membrane constitutes the diffuse glomerulosclerosis seen by the light microscope. Nodular glomerulosclerosis (The Kimmelstiel-Wilson nodule) may result from focal thickening in the diffusely thickened basement membrane. This is in accordance with Bell's original idea that the nodular lesions are derived from the diffuse lesions (11). Since diabetic glomerulosclerosis has thus been clearly shown to be a lesion of the basement membrane it should no longer be described in light microscopic studies as "intercapillary" or

"intracapillary".

The finding that in diabetes the nephron is involved in the very early stages of the disease must add considerable weight to the theory that some of the renal lesions are in fact an integral part of the disease process rather than a complication of it. The fact that the same types of changes were found in the juvenile onset and the maturity onset types of diabetes excludes the idea that a specific type of diabetes is characterised by the development of nephropathy as has been suggested (51). In this respect it would be of great interest to examine renal tissue from those individuals classified as "pre-diabetics" to see whether an alteration in the renal ultrastructure preceeds the clinical onset of hyperglycaemia and glycosuria or appears only in association with it. Also, it would be very important to see whether diabetic patients whose hyperglycaemia is due to a pathological destruction of the pancreatic islets (e.g. haemochromatosis, chronic pancreatitis) will show these electron microscopical abnormalities in the nephron or whether the changes are specific to the "idiopathic" type of diabetes. Finally, it would be most important to study whether these ultrastructural abnormalities regress with proper insulin replacement therapy, in the same way as the abnormality in glucose metabolism responds to insulin, or whether the metabolic error that provokes them does not respond to insulin. If this be so, the eradication of diabetic glomerulosclerosis must depend largely upon the elucidation of its basic metabolic defect and the therapeutic agent that corrects it, and not, or not only upon the efficient treatment of hyperglycaemia and glycosuria.

II. Light and Electron Microscopic Study of Prednisolone-induced Renal Lesions.

Introduction.

While studying the effect of cortisone on the renal lesions produced by anaphylactic hypersensitivity in rabbits, Rich, Berthrong and Bennett (43) observed dilatation of glomerular capillary loops, focal necrosis of cells of loops and formation of large hyaline masses in the tufts recalling the focal hyaline masses in diabetic glomeruli and in some cases of disseminated lupus erythematosus. In ACTH-treated animals, no such lesions were observed. Similar lesions were later produced in rabbits by Friedenwald (26), McLean et al (36), Becker (7), Germuth (27), Bloodworth and Hamwi (15) and Wilens and Stumpf (49). Cortisone administration has not been shown to produce similar lesions in other species, and, according to Becker (7) ACTH does not cause the lesions unless the rabbits have been previously rendered diabetic by alloxan. Friedenwald (26) and Becker (7) have also shown that retinal microaneurysms developed if the animals were pretreated with alloxan. Bloodworth and Hamwi (16) were able to produce these lesions also with hydrocortisone but not with DOCA which, however, was noted in one alloxan diabetic rabbit to produce the typical K.W. nodules after its administration for three months. According to these authors, cortisone glomerular lesions are similar to human diabetic "exudative lesions". They believe that these lesions are thrombi composed of mucopolysaccharide and lipid within dilated capillaries. Wilens and Stumpf (49) have shown a sequence of events that develops after cortisone administration to rabbits; increase in the neutral fat content of the blood, irregular dilatation of glomerular capillaries, especially theperipheral portions of the loops,

and later, occlusion of the dilated capillaries with fused masses of erythrocytes and lipid-rich plasma which is eventually converted into a wax-like material.

In 1959, Farquhar et al (24), studying the electron microscopic appearances of glomeruli in diabetic human subjects suffering from clinically evident nephropathy arrived at the conclusion that the "exudative lesions" consist of electron dense material "fibrinoid" deposited subendothelially between the endothelial cytoplasm and the thickened basement membrane and not intraluminally.

It was thought that a further study of these corticosteroid-induced glomerular lesions, including electron microscopy, might throw a light on their anabgy to human diabetic exudative lesions and might be of value in elucidating further their pathogenesis.

Material.

Three adult rabbits were given 1 mg. prednisolone four times weekly in a single intramuscular injection for a period of 12 weeks. The urine was collected once weekly and examined for sugar and protein.

The rabbits were sacrificed on the last day of prednisolone administration. Both kidneys were bisected; one half of each kidney was fixed in corrosive formol and later examined by multiple staining techniques: haematoxylin and eosin, PAS, Picro-Mallory, Masson's trichrome, methyl violet and frozen sections were stained by Sudan IV for fat. The other half was prepared for electron microscopy as previously described.

Kidneys from three adult normal rabbits were studied by the same methods and served as controls.

Results.

All the rabbits lost a slight to moderate degree of weight from 10% to 45% of their original weight. They all developed glycosuria, one of them immediately following prednisolone administration; in the others, the glycosuria was noted in the second or third week of treatment. The glycosuria tended to diminish and to disappear in the sixth or seventh week of treatment.

All the rabbits developed proteinuria at about the sixth week of steroid administration and this persisted until the animals were sacrificed. They also showed haematuria in the last three weeks.

The rabbits were not given potassium supplements; they gradually showed the clinical manifestations of potassium deficiency, weakness and flaccidity of the muscles amounting to complete flaccid paralysis of the hind limbs in one of them.

Light Microscopic Findings.

Changes were observed in the glomeruli and tubules of the prednisolone treated rabbits while the kidney from the control animals were entirely normal.

A. Glomerular Lesions.

The glomerular tufts were enlarged and the capillaries were conspicuously dilated (Fig. 98). Many capillaries were markedly dilated, reaching aneurysmal proportions (Fig. 99).

Numerous glomeruli showed hyaline fibrinoid lesions. In the sections stained with haematoxylin and eosin (Fig. 100) they appeared sharply defined,

highly eosinophilic and glossy. The lesions were characteristically situated at the periphery of thetuft. They were crescentic in shape, sometimes round or oval and were completely acellular. Fine to coarse vacuoles were frequently present. On closer scrutiny, smaller fragments of similar material could be seen in various locations within the loops of the glomerular tufts.

In Masson's trichrome stain and with Picro-Mallory's stain, the lesions stood out in the form of brilliant red masses, characteristic of the so-called "fibrinoid" material while the rest of the glomerulus stained normally (Fig. 101). Those hyaline fibrinoid lesions gave the impression of being dense coagula arranged in the form of crescents or globules found predominantly at the periphery of the glomeruli and bulging into Bowman's space. In extreme cases, a partial or almost complete transformation of a glomerular tuft into a mass of vivid red material could be observed. It was evident that this lesion was not located in Bowman's space, since it was completely covered by a well preserved visceral layer of the capsular epithelium, and separated by a narrowed but empty subcapsular space from the parietal layer of cells.

These lesions often had a high fat content (Fig. 102). The lipids were usually finely dispersed, giving a diffuse orange stain with Sudan IV.

These hyaline fibrinoid lesions were sometimes seen in duplicate and occasionally even in triplicate in the same glomerulus. In addition, the glomerular tufts were often significantly deficient in nuclei. Bowman's space was frequently occupied by a variable mixture of proteinous material, fibrin, blood and fat.

The cells lining Bowman's capsule were sometimes swollen, vacuolated,

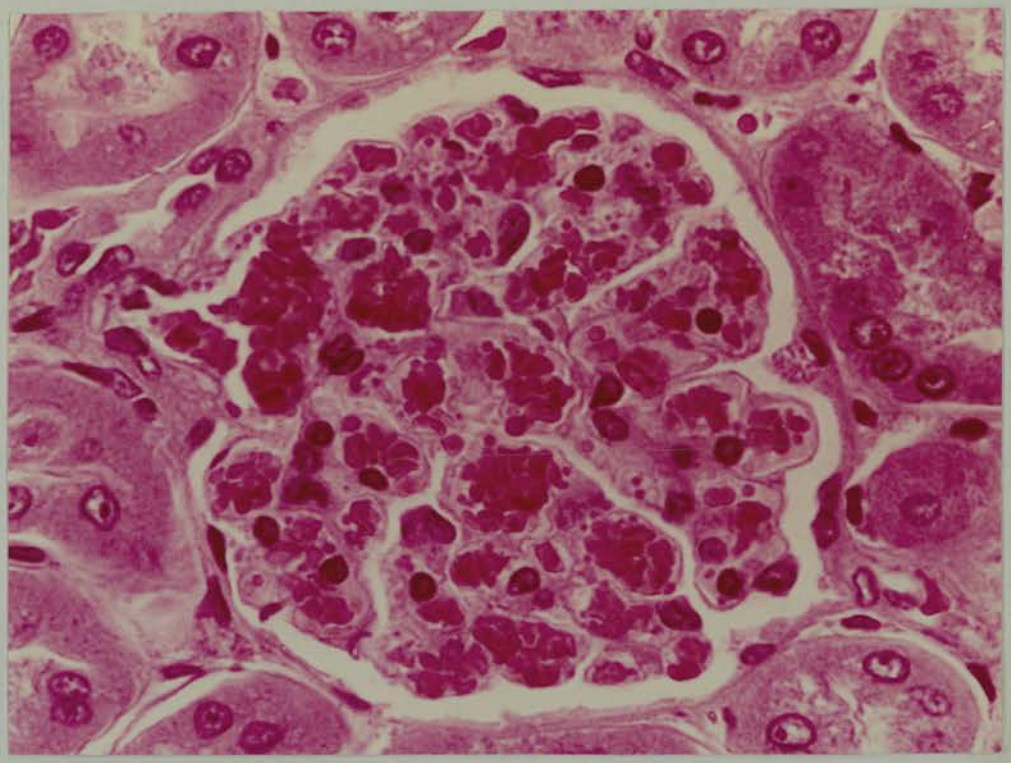


Fig. 98. Glomerulus from a Prednisolone-treated rabbit. Most capillaries are dilated and full of red blood corpuscles. Note the scarcity of nuclei in the tuft. Hx & Eo. x 800

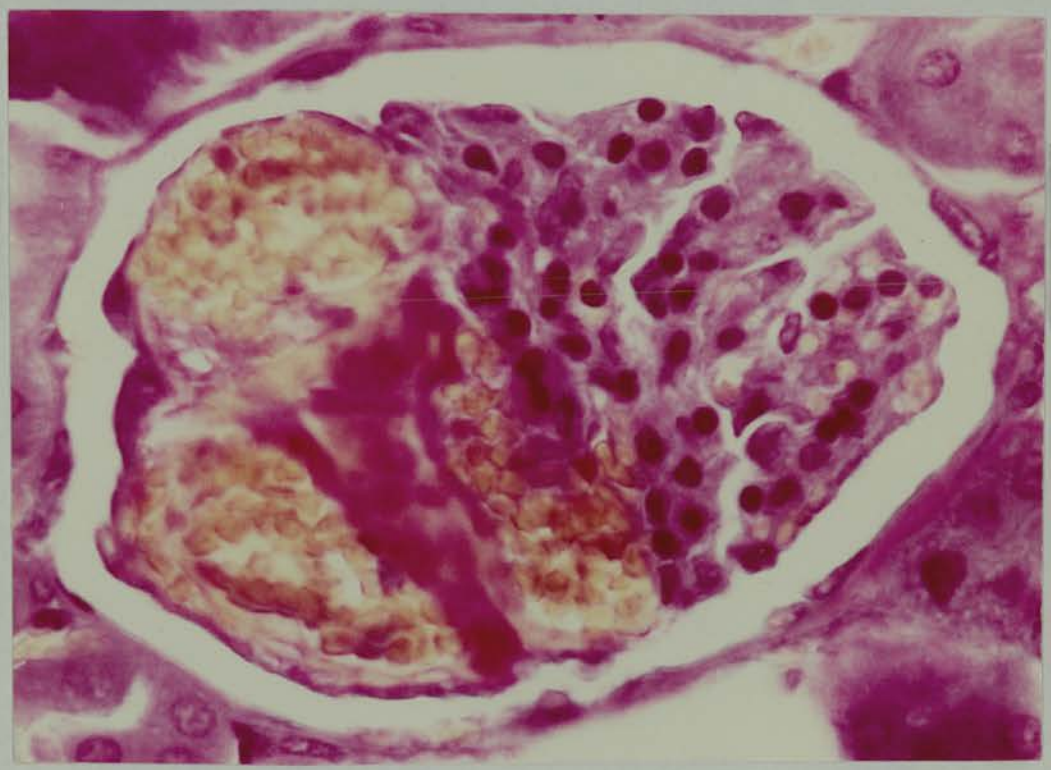


Fig. 99. Glomerulus from a Prednisolone-treated rabbit. A capillary microaneurysm occupies more than half of the tuft. Picro-Mallory x 900

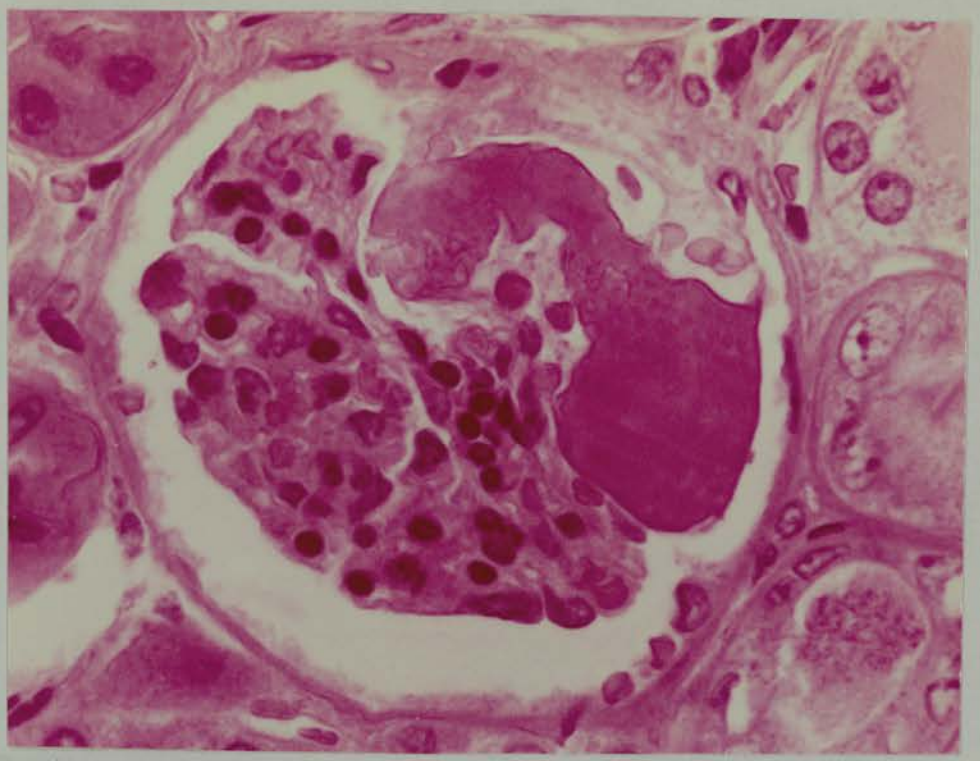


Fig. 100. Glomerulus from a Prednisolone-treated rabbit, a crescentic, highly eosinophilic, glossy "fibrinoid" lesion is seen at the periphery with a dilated, patent capillary above it. Hx & Eo. x 900

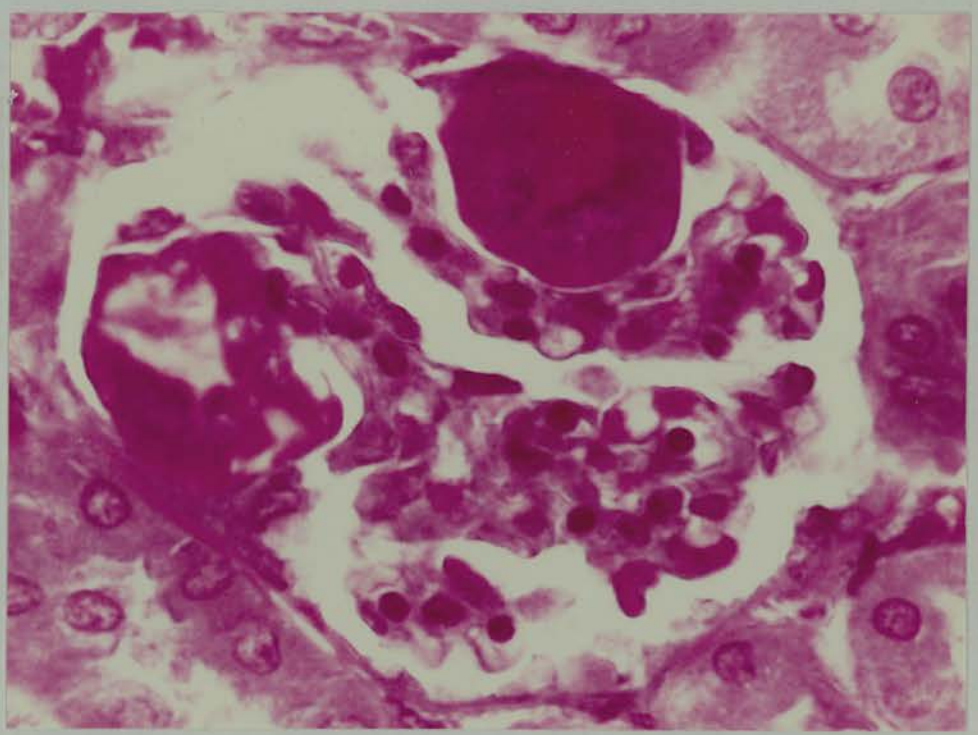


Fig. 101. Glomerulus from a Prednisolone-treated rabbit. Two rounded lesions are seen at the periphery of the tuft taking the red colour characteristic of "fibrinoid" in this staining technique. The lesion on the left side is vacuolated.
Picro-Mallory x 1000

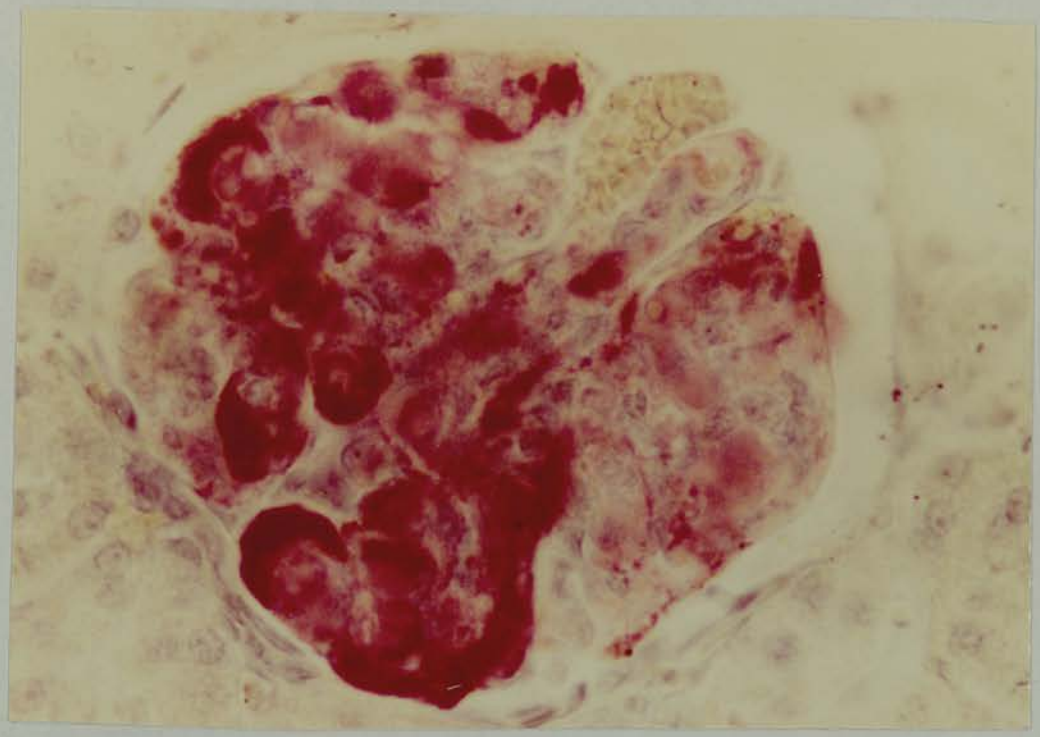


Fig. 102. Glomerulus from a Prednisolone-treated rabbit. Note the sudanophilia of the "fibrinoid" lesions. Sudan IV counterstained with Hx x 700

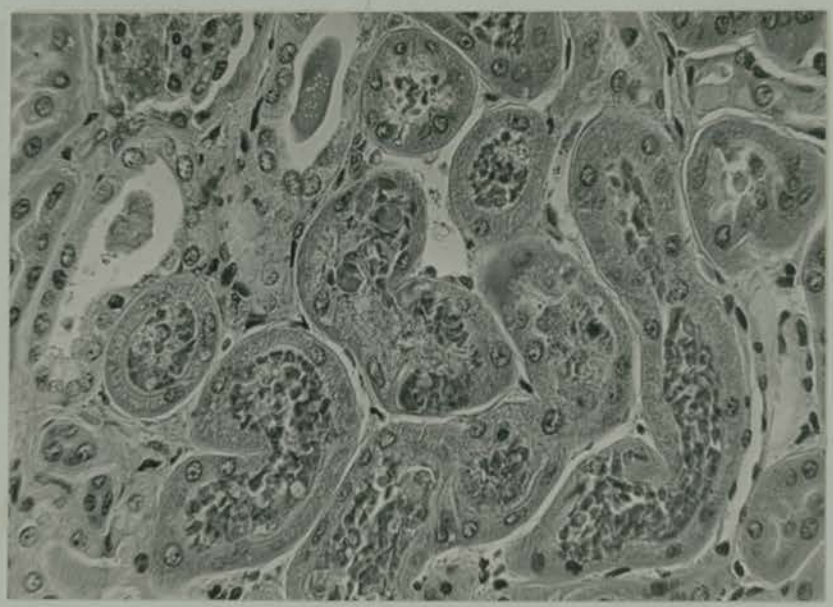


Fig. 103. Proximal convoluted tubules from a prednisolone-treated rabbit. The lumina are filled with red blood corpuscles. x 275.

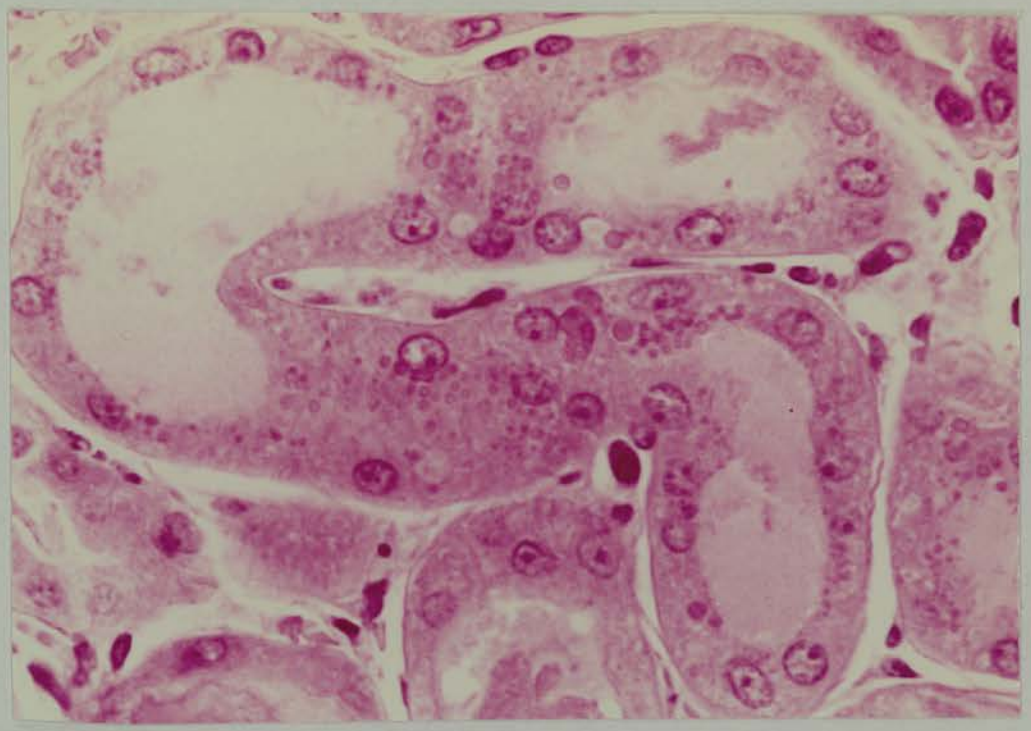


Fig. 104. Proximal convoluted tubules from a prednisolone-treated rabbit. Note the hyaline droplets within the cytoplasm. Hx & Eo. x 850.

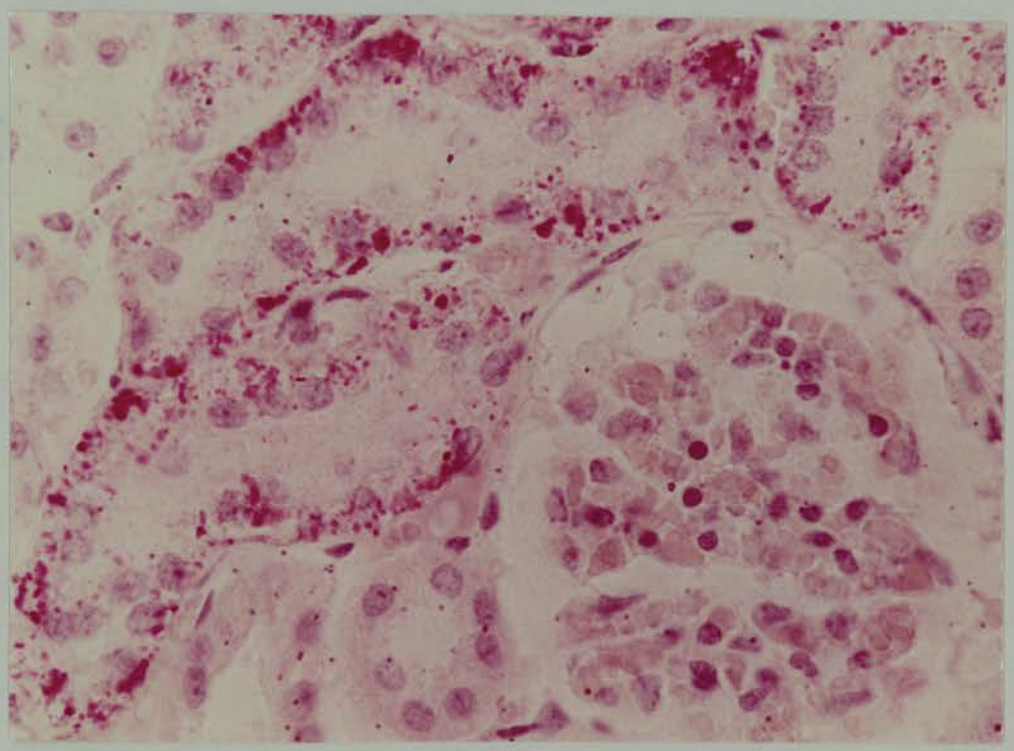


Fig. 105. Distal convoluted tubules from a prednisolone-treated rabbit. Note the fatty droplets in the basal part of the cell.
Sudan IV counterstained with Hx. x 600

necrotic and desquamated. Fibrinoid lesions were sometimes observed lining Bowman's capsule and giving an appearance identical with the "capsular drop" seen in human diabetic glomeruli.

B. Tubular Lesions.

Many tubules contained in their lumina hyaline eosinophilic casts, blood, fat, necrotic cells and a granular material (Fig. 103). The cells of some proximal tubules contained hyaline droplets in their cytoplasm (Fig. 104). Fat droplets were also often seen, particularly in the cytoplasm of the distal tubules (Fig. 105). An occasional necrotic, deeply eosinophilic cell could be seen in the lining of the tubules, particularly in the collecting tubules.

Electron Microscopic Findings.

A. Glomerular Lesions.

In the experimental animals, the initial and constant alteration was uniform dilatation of almost all glomerular capillaries. One or two capillaries in some glomeruli were disproportionately enlarged (Fig. 106). The endothelial fenestrae in the dilated capillaries appeared larger in size than normal, having an average diameter of 1200 Å, and in the markedly dilated capillaries the fenestrae measured as much as 2100 Å indiameter (Fig. 107).

The most characteristic change noted was the deposition of deeply osmiophilic material in the glomerular capillaries. In grossly involved capillaries the material appeared to fill the lumen almost completely (Fig. 108).

When earlier degrees of the lesion were studied, this material, which is identifiable with the "fibrinoid" deposit described above by light microscopy, appeared to be accumulating between the basement membrane proper and the endothelium (Fig. 109), or to be deposited within the endothelial cytoplasm giving it a dense structureless appearance (Fig. 110).

Sometimes, the "fibrinoid" was noted to permeate the basement membrane which itself was increased in amount, and this resulted in a thick mass of "fibrinoid" and basement membrane material mixed up together, forming the wall of the glomerular capillary (Fig. 111).

The epithelial cells were in many respects normal in appearance, though some showed excessive vacuolation of the cytoplasm. This, together with the finding of hyaline "protein-absoprtion" droplets in the cytoplasm of the epithelial cells indicated that they were very active, trying to cope with the excessive filtration of protein and other colloidal plasma constituents that have by-passed the glomerular filter. Some epithelial cells, however, had a dense osmiophilic appearance with disappearance of the nucleus and of all details of the intracytoplasmic organelles (Fig. 112). The trabeculae and the pedicels of these epithelial cells shared in this dense osmiophilic appearance.

Apart from the osmiophilia, the pedicels were usually normal, though smearing of the external surface of the basement membrane due to confluence of pedicels and loss of their normal arrangement was occasionally observed (Fig. 107).

The basement membrane of Bowman's capsule was normal in thickness and appearance. Its lining cells were frequently normal, though an occasional cell was very densely osmiophilic with disappearance of nuclear and mito-

chondrial details (Fig. 113).

B. Tubular lesions.

The most characteristic change noticed was selective necrosis of tubular cells. This was seen in proximal tubular cells, thin and thick segments of Henle's loops, distal tubules and collecting tubules, particularly in the collecting tubules. Various stages of this necrotic process were observed. The earliest change was increased density of the cytoplasm, veiling the intracellular contents, associated with a gradual shrinkage of the cell (Fig. 114). The density of the cytoplasm progressively increased till it completely obscured the intracellular contents. This was associated with a gradual vacuolar degeneration of the nucleus and cytoplasm and progressive shrinkage in size (Fig. 115). The vacuolar change was more noticeable in the cells of the collecting tubules than in other parts of the nephron (Fig. 116).

The selective increased density and progressive shrinkage of the cells exhibited very clearly the interdigitating trabeculae. Fine short secondary processes were also seen, arising from the lateral margins of the necrotic trabeculae (Fig. 117, 118, 119). The shrunken necrotic cell and its processes showed marked electron density and appeared similar to the "fibrinoid" seen in the glomeruli.

Hyaline bodies, similar to those seen in the glomerular epithelial cells, were present in the cells of the proximal convoluted tubules. These probably indicate an excessive pinocytic activity by these cells of filtered protein.

Cellular debris and very electron dense globules were seen in the tubular lumina (Fig. 120); these strongly osmiophilic bodies are probably

Fig. 106. Glomerular capillary from a prednisolone-treated rabbit. Note the marked dilatation. x 4,000

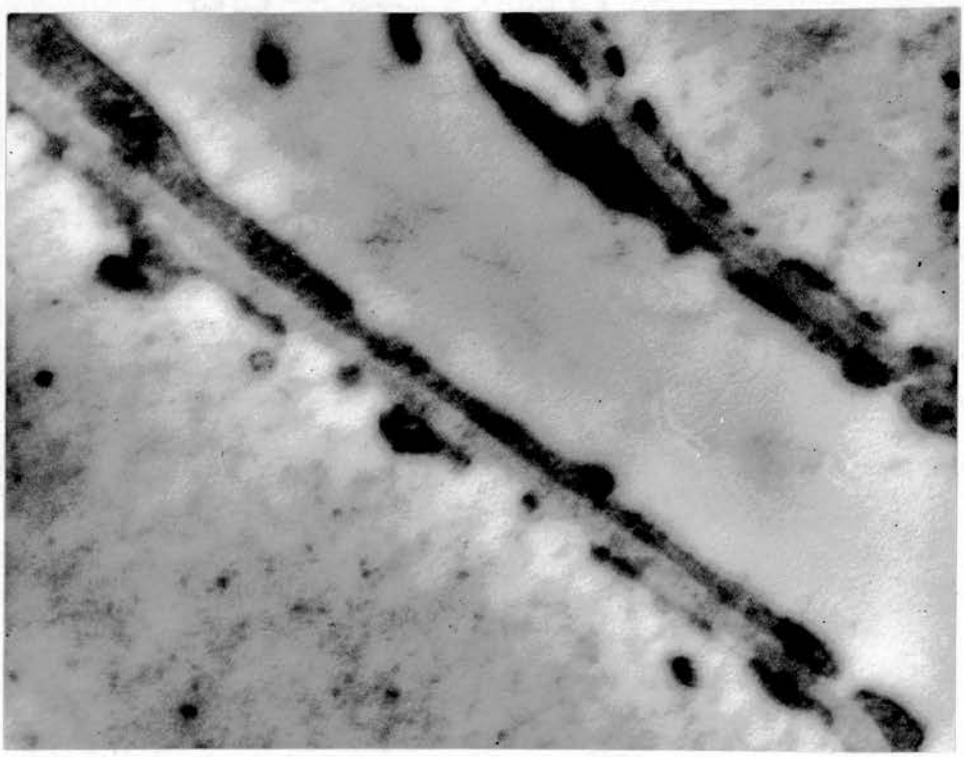


Fig. 107. Two glomerul ar capillary walls from a prednisolone-treated rabbit. Note that the endothelial fenestrae are wide and that the normal pedicel arrangement has been replaced by a film of epithelial cytoplasm. The basement membrane is of normal thickness. x 45,000

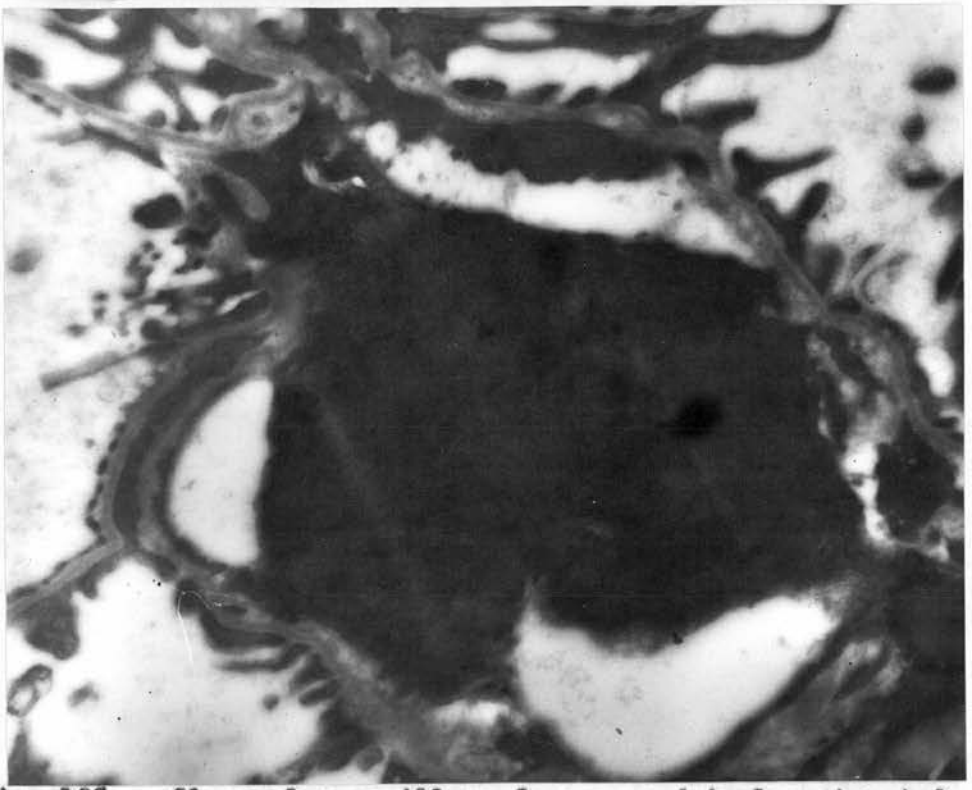


Fig. 108. Glomerular capillary from a prednisolone-treated rabbit almost completely occluded by a densely osmiophilic structureless deposit. No thickening of the basement membrane is apparent. x 19,000

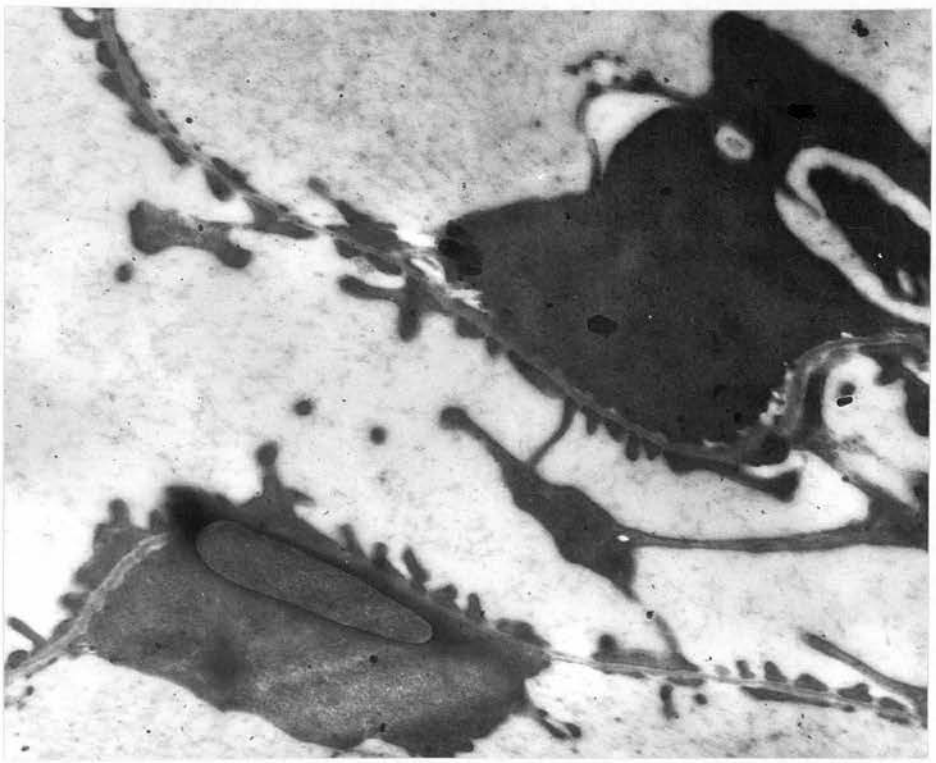


Fig. 109. Two glomerular capillaries from a prednisolone-treated rabbit. Note the densely osmiophilic, structureless deposits, which appear clearly subendothelial in position in the upper one. The basement membrane is of normal thickness. x 15,000

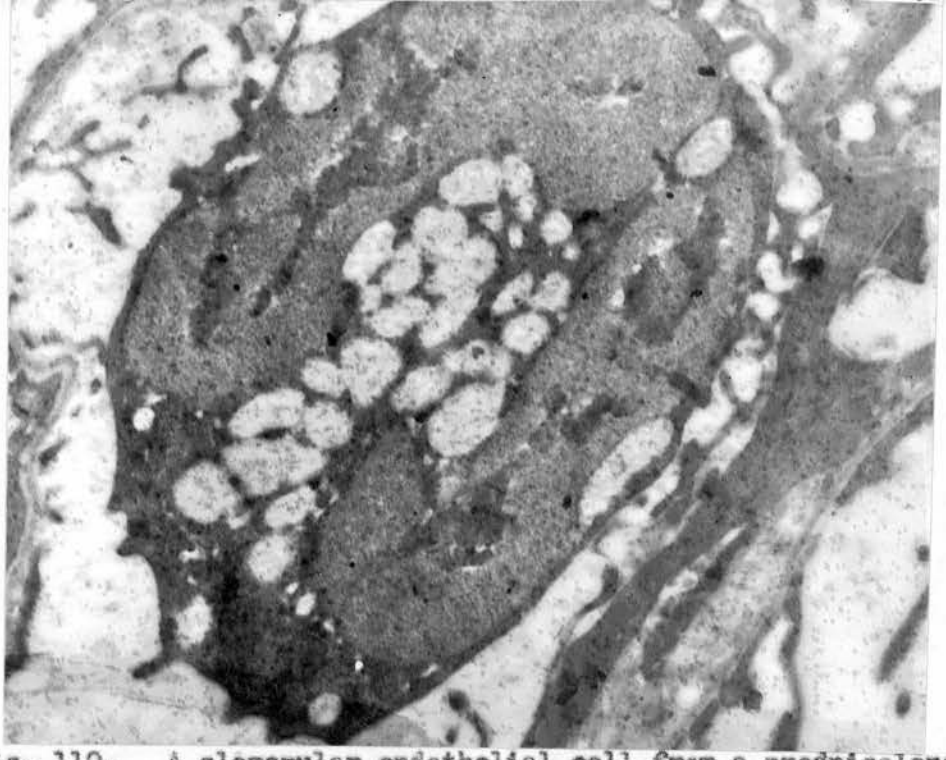


Fig. 110. A glomerular endothelial cell from a prednisolone-treated rabbit. Note the osmiophilic deposits in the cytoplasm, the vacuolar changes in the nucleus and the normal thickness of the capillary basement membrane. x 24,000

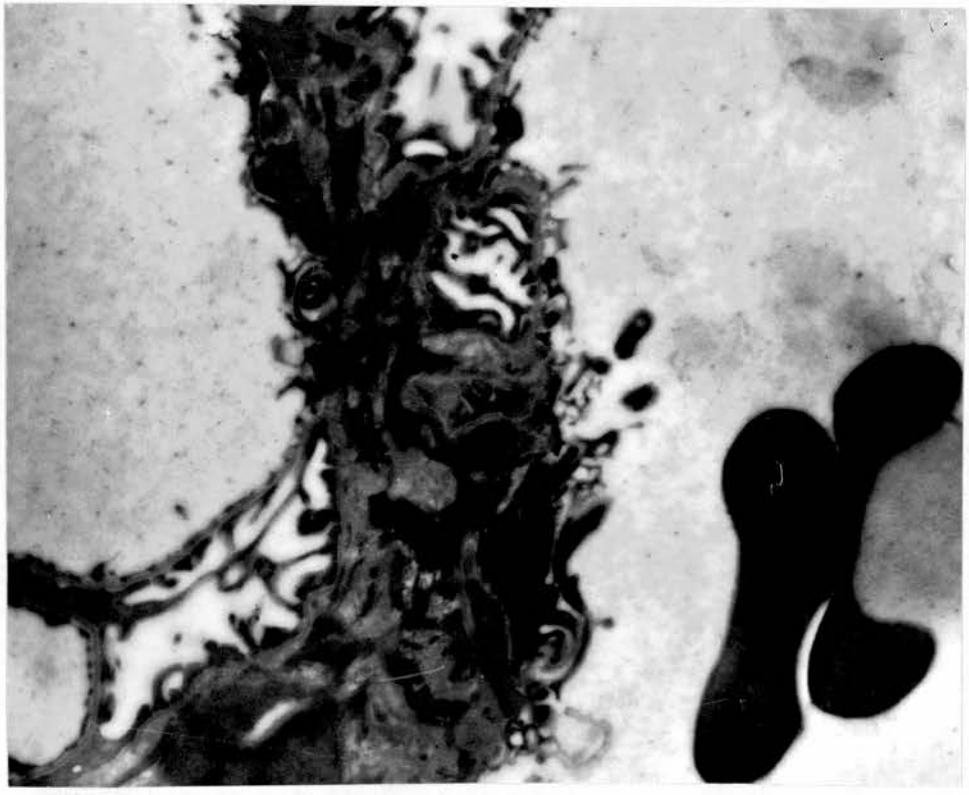


Fig. 111. Three adjacent glomerular capillaries from a prednisolone-treated rabbit. The densely osmiophilic deposit is intermingled with the basement membrane in a complex structure, in which masses of basement membrane like material can be seen. x 12,000

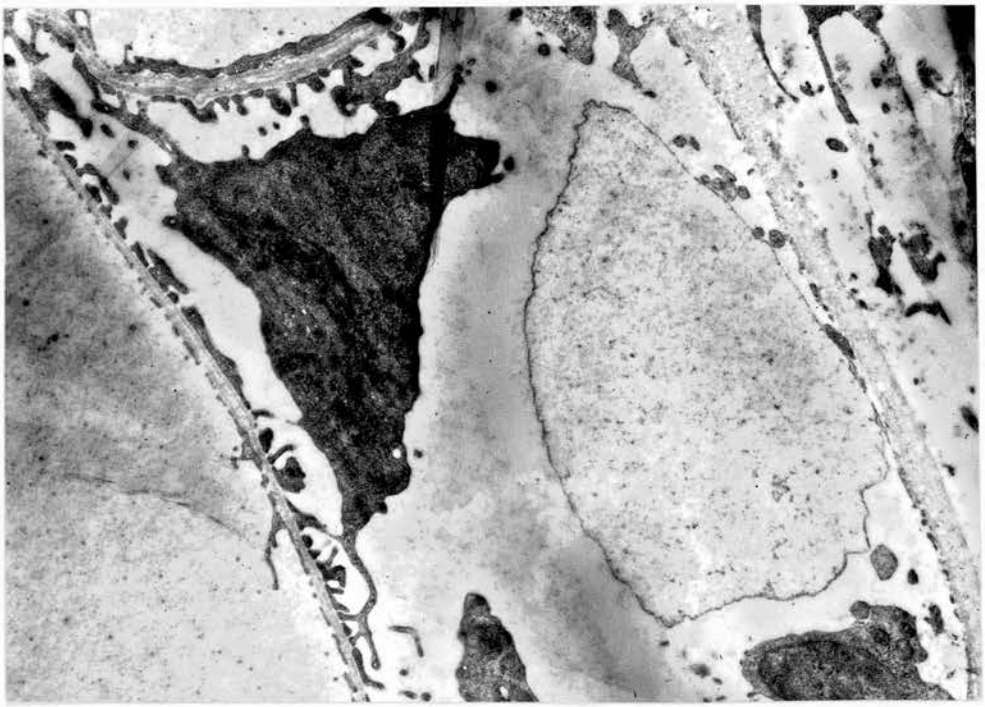


Fig. 11 2. Peripheral part of a glomerulus from a prednisolone-treated rabbit. The epithelial cell cytoplasm is densely osmiophilic, obliterating the details of the intracellular structures. A large globule containing plasma-like material is seen in Bowman's space. x 9,000

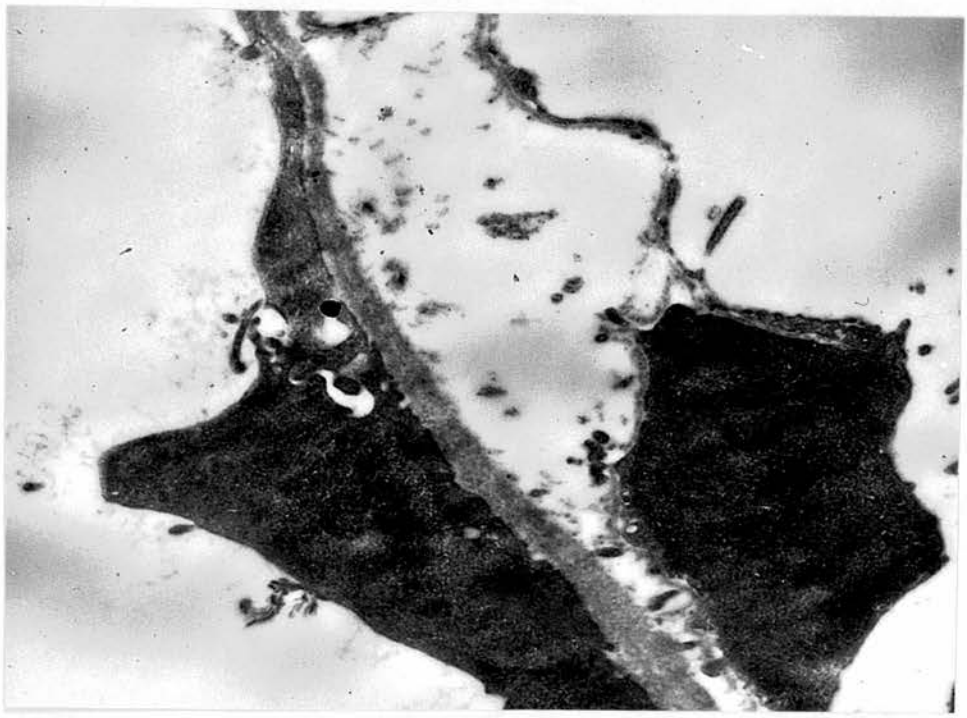


Fig. 113. Bowman's capsule from a prednisolone-treated rabbit. The lining cell-cytoplasm is densely osmiophilic, and the cell looks like a "fibrinoid" deposit hanging from the capsule. Another "fibrinoid" deposit is seen in the wall of an adjacent intertubular capillary. x 15,000

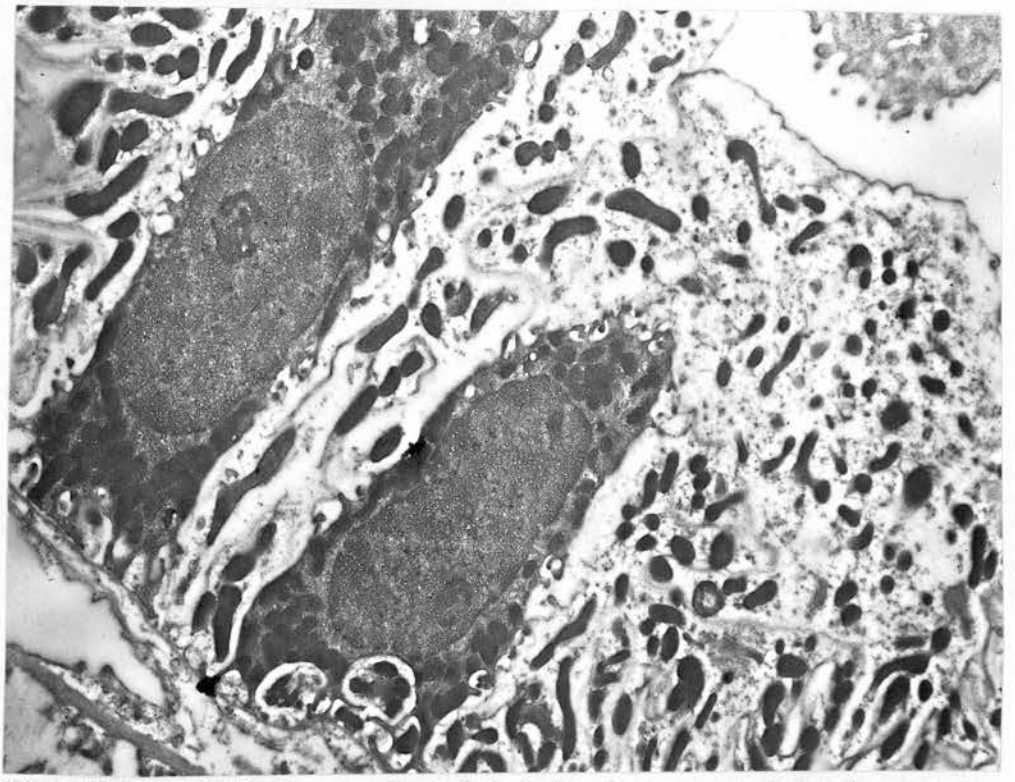


Fig. 114. Distal convoluted tubule from a prednisolone-treated rabbit. Two cells are shrunken away from the lumen, their mitochondria crowded together and their cytoplasm more osmiophilic than the surrounding normal cells. The basement membrane is normally thin. x 6,000

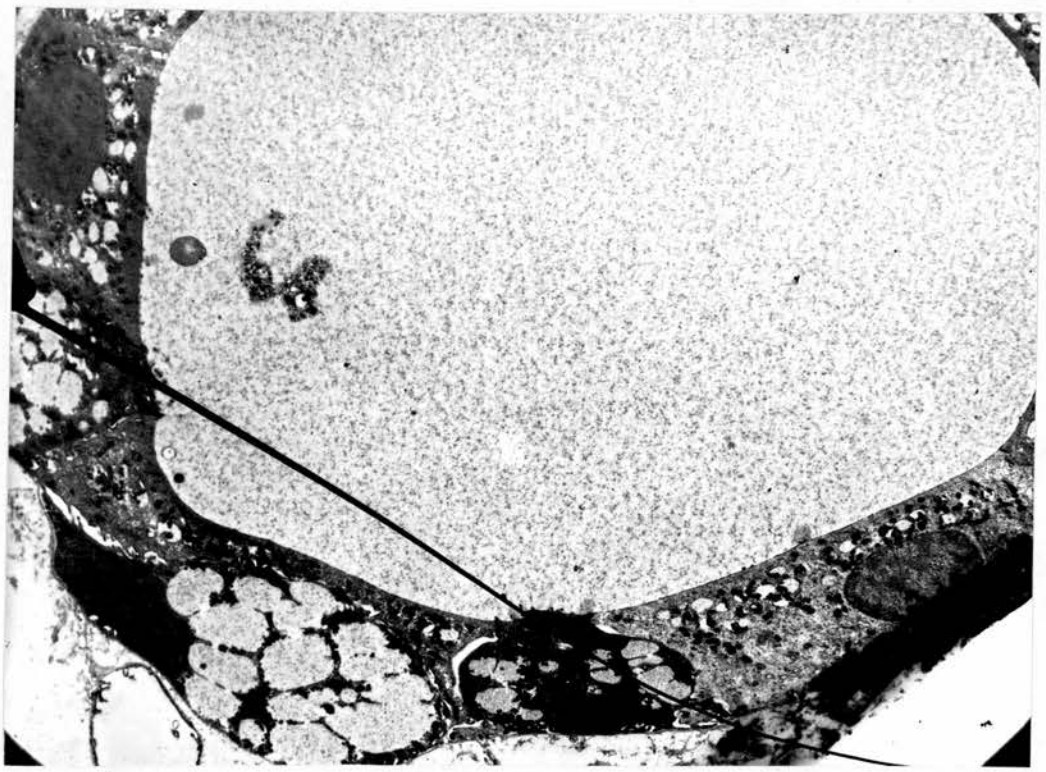


Fig. 115. Cortical collecting tubule from a prednisolone-treated rabbit. The lumen is very dilated and the lining cells show varying degrees of degenerative changes. x 2,500

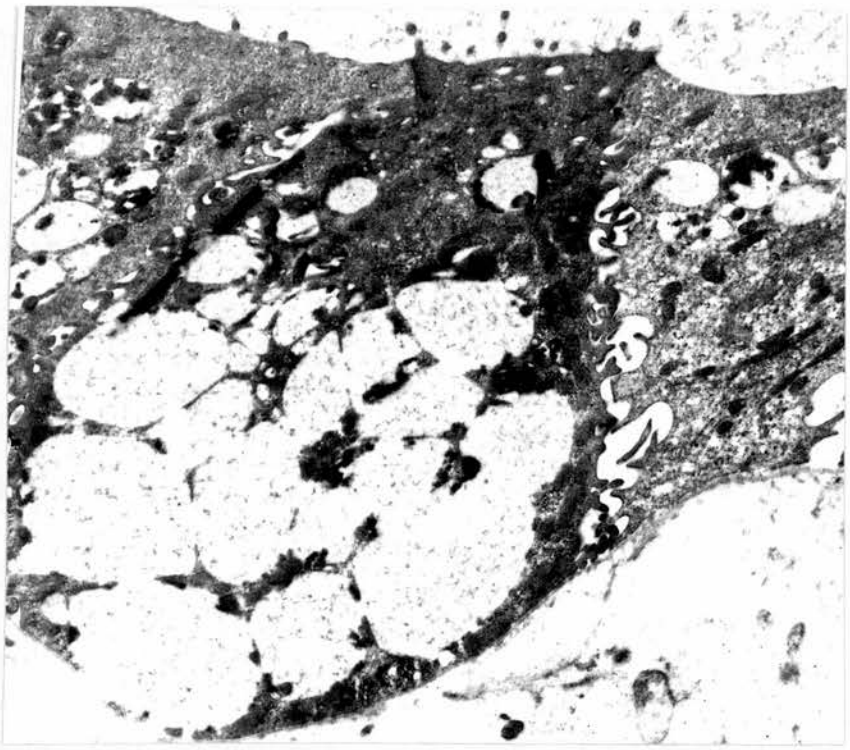


Fig. 116. Cortical collecting tubule cell from a prednisolone-treated rabbit. The cell is shrunken, its cytoplasm is vacuolated and more osmiophilic than normal. x 6,000

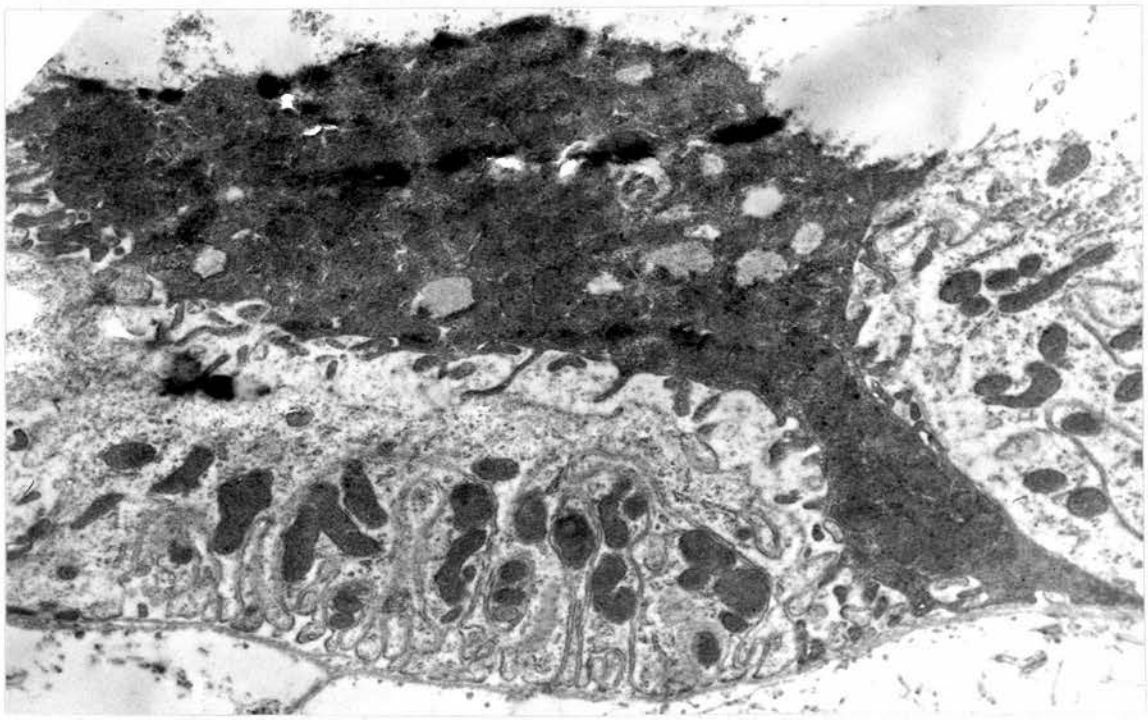


Fig. 117. Necrotic cell in the thick segment of the ascending limb of the loop of Henle in a prednisolone-treated rabbit. Note the thick primary process from the lower right corner of this cell wedged in between two normal cells and the fine secondary processes arising from it and from the rest of the cell surface. x 12,000

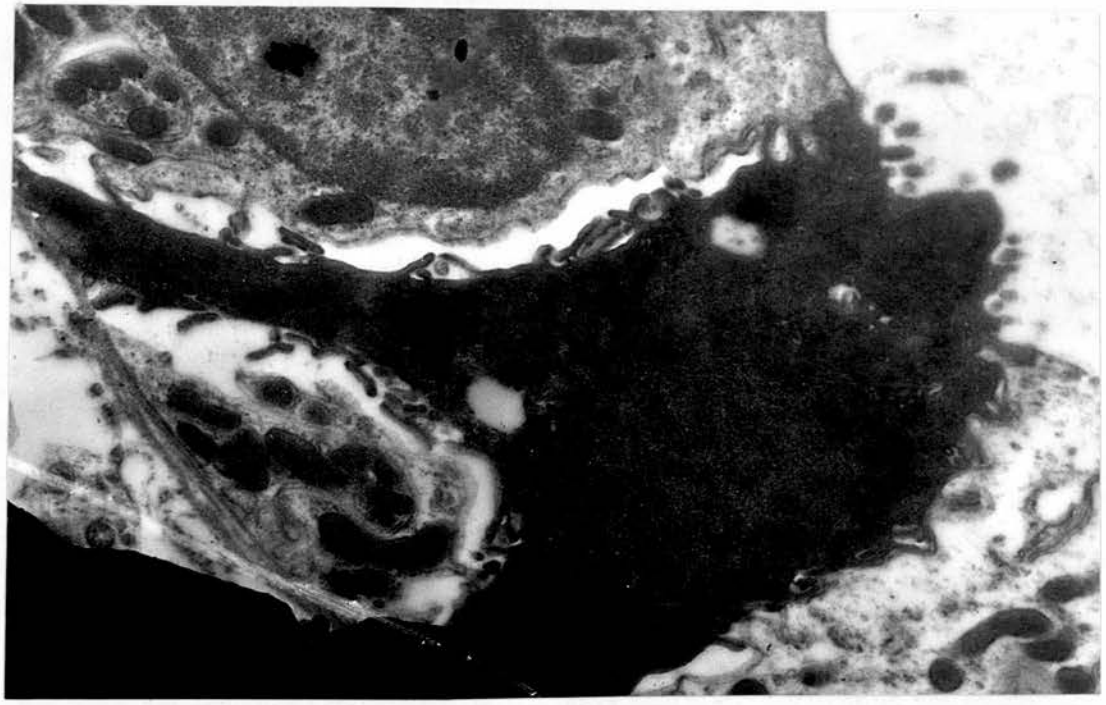


Fig. 118. Necrotic, densely osmiophilic cell in a cortical collecting tubule in a prednisolone-treated rabbit. Note the thick primary and the fine secondary processes. x 12,000

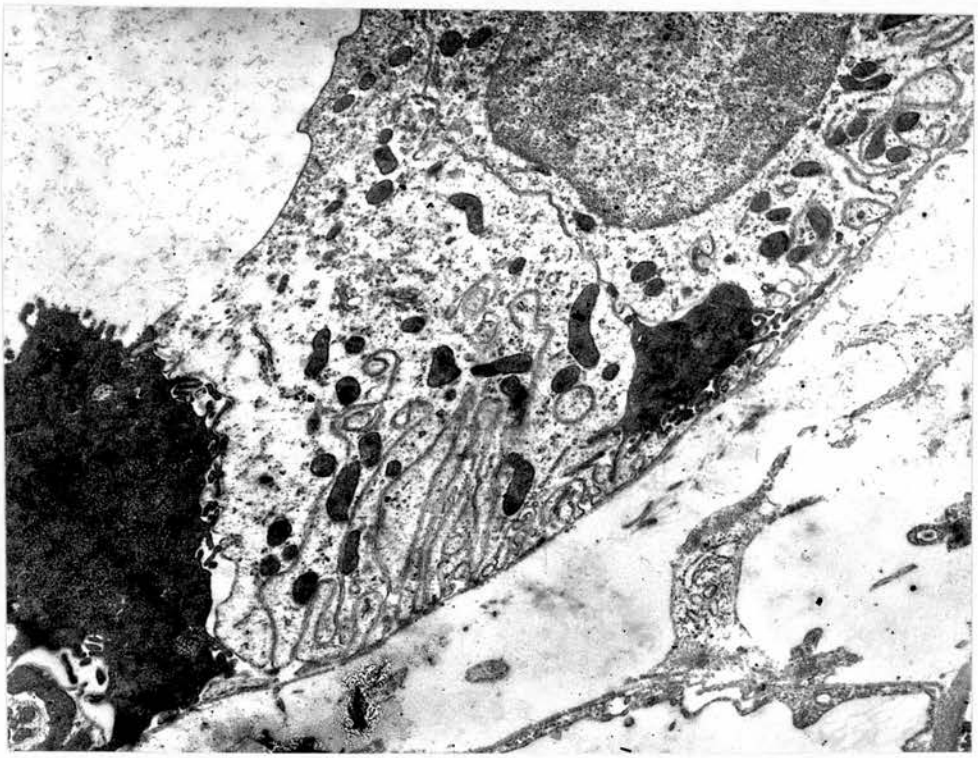


Fig. 119. Cortical collecting tubule from a prednisolone-treated rabbit. The densely osmiophilic process wedged in between the two normal cells apparently belongs to the necrotic, densely osmiophilic cell on the left. x 9,000

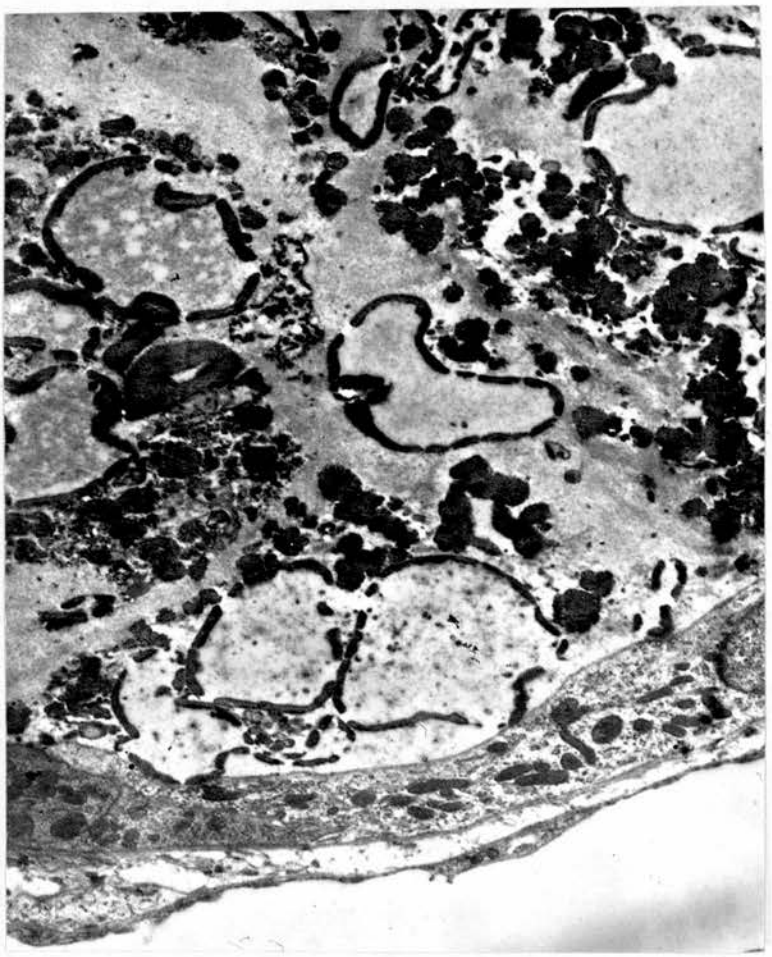


Fig. 120. A medullary collecting tubule from a prednisolone-treated rabbit. The lumen is very dilated and full of strongly osmiophilic globular bodies, mitochondria and other cellular debris. x 6,000

lipoid in nature.

Many distal convoluted tubules and collecting tubules were also markedly dilated (Fig. 120).

DISCUSSION.

In this study, it has been shown that prednisolone, like cortisone and cortisol, but unlike ACTH and DOCA, is capable of producing dilatation of glomerular capillaries, capillary micro-aneurysms and hyaline fibrinoid lesions in the glomeruli of rabbits. These lesions are exactly similar to human exudative diabetic lesions. By light microscopy, both types of lesion appear to develop in the lumina of dilated glomerular capillaries, particularly the peripheral loops and usually have a crescentic shape when small. Both types of lesion stain brilliant red, characteristic of what is known as "fibrinoid" by Masson's trichrome and Picro-Mallory techniques, and both frequently have a high fat content. By the electron microscope, the human (24) and the steroid-induced lesions are seen to have the same homogeneous dense osmiophilic appearance, and the same location in the glomerular capillary wall. The steroid-induced lesions, however, are different from diabetic diffuse and nodular glomerulosclerosis. These latter lesions stain green with Masson's trichrome and blue with Picro-Mallory stains and are devoid of fat. Diabetic glomerulosclerosis by the electron microscope appears as marked thickening of the capillary basement membrane. Prednisolone was not noted to produce any thickening in the basement membrane itself and the "fibrinoid" is quite different in appearance from basement membrane material. Moreover the steroid lesions have not progressed to glomerulosclerosis even after such long term administration. However, the

production of steroid diabetes in the three rabbits by prednisolone and the concurrent production of proteinuria and glomerular lesions similating human diabetic nephropathy would appear to relate these lesions to diabetes further.

There has been a great deal of speculation concerning the nature of these steroid lesions. Wilens and Stumpf (49) have stressed the importance of lipid in the production of the cortisone lesions. They pointed out that cholesterol, phospholipid and neutral fat were all elevated by the administration of cortisone. Cortisone also causes an elevation of the lipoprotein component in the S_f 10-30 class. Moreover, similar lesions have been produced by other methods of interfering with lipid metabolism; Hartroft (31) was able to produce them in choline-deficient rats.

Human diabetic fibrinoid lesions are probably lipoprotein in nature (44). A disturbed lipoprotein metabolism has been found in diabetics with nephropathy. (32). Also, increased adrenal cortical activity has been demonstrated in those patients (21). Therefore, since an alteration in lipoprotein metabolism is involved in the pathogenesis of the fibrinoid lesions, and since changes in adrenal cortical activity are associated with alterations in the serum lipoproteins, the hypothesis that adrenal cortical hyperactivity plays a role in the development of these lesions, would seem quite plausible. On the other hand, adrenal cortical hyperfunction is probably not directly related to the development of diffuse and nodular glomerulosclerosis.

As these rabbits were very probably deficient in potassium, and possibly also in magnesium, as a result of the prolonged non supplemented administration of the corticosteroid, it is impossible to exclude the

possibility that some of the tubular changes observed were due to this factor. The hyaline droplets and fatty droplets in the tubular cytoplasm most probably reflect a hyperactivity on the part of the tubular cells to pinocytose the excessive amounts of protein and fat that have passed through the defective glomerular capillaries. On the other hand the degeneration, necrosis and desquamation of the tubular cells is most likely to be the result of some cation deficiency, potassium and magnesium being the main intracellular cations on which cellular integrity must surely depend. The exact nature and explanation of these lesions can only be solved by repeating the experiment of administration of prednisolone and giving sufficient potassium and magnesium supplements to prevent any kaliopenia or magnesium deficiency, and by putting animals on a potassium deficient and on a magnesium deficient diet and examining their kidneys by the electron microscope to see the pure effects of the cation depletion, without the metabolic effects of the steroid.

The selective necrosis of tubular cells seen in these rabbits showed very clearly for the first time the validity of Rhodin's (42) hypothesis as to the manner of connection between adjacent tubular cells. It proved the existence of interdigitating processes arising from the lateral sides of the basal half of the cells. In addition, very fine secondary processes were seen arising from these, in a manner similar to the arrangement of trabeculae and pedicels of the glomerular epithelial cells. Rhodin could not define these latter secondary processes.

REFERENCES.

1. Adlersberg, D., Wang, C.J., Rifkin, H., Berkman, J., Ross, G. and Weinstein, C.
Serum lipids and polysaccharides in diabetes mellitus.
Diabetes 5: 116, 1956.

2. Allen, A.C.
So-called intercapillary glomerulosclerosis. Lesions associated with diabetes mellitus. Morphogenesis and significance.
Arch. Path., 32: 33, 1941.

3. Anderson, G.S.
The pathogenesis of diabetic glomerulosclerosis.
J. Path. Bact., 67: 241, 1954.

4. Ashton, N.
Proc. 16th International Congress Ophthal.
LONDON, 1, 411, 1950.

5. Barr, D.P. and Russ, E.M.
Protein-lipid relationship in diabetes mellitus.
Trans. Ass. Amer. Phys., 64: 297, 1951.

6. Barrie, H.J., Askanazy, C.L. and Smith, G.W.
More glomerular changes in diabetics.
Canad. med. Assoc. J., 66: 428, 1952.

7. Becker, B.
Diabetic Retinopathy.
Ann. Int. Med., 37: 273, 1952.

8. Becker, B., Allen, R., Winter, F.C., Maengwyn-Davies, G., and Friedenwald, J.S.
The role of the adrenal cortex and vitamin B12 in diabetic retinopathy.
Amer. J. Ophthal., 38: 53, 1954.

9. Becker, B., Lang, C.A. and Chow, B.F.
Vitamin B12 excretion of diabetic retinopathy.
J. Clin. Nutrition. 1; 417, 1953.

10. Becker, B., Maengwyn-Davies, G., Rosen, D., Friedenwald, J.S. and Winter, F.C.
The adrenal cortex and B-vitamins in diabetic retinopathy.
Diabetes, 3: 175, 1954.

11. Bell, E.T.
PHILADELPHIA, Lea and Febiger, 1946.

12. Bergstrand, A. and Buch, H.
Electron microscopic investigations on the glomerular lesions in diabetes mellitus (diabetic glomerulosclerosis).
Lab. Investigation, 6: 293, 1957.

13. Berkman, J., Rifkin, H. and Ross, G.
The serum polysaccharides in diabetic patients with and without degenerative vascular disease.
J. Clin. Invest., 32: 415, 1953.

14. Berkman, J., Ross, G. and Rifkin, H.
Serum polysaccharides in diabetic and nondiabetic patients with degenerative vascular disease.
Bull. N.Y. Acad. Med., 30: 316, 1954.

15. Bloodworth, J.M.B. Jr., and Hamwi, G.J.
Histopathology of experimental glomerular lesions simulating human diabetic glomerulosclerosis.
Amer. J. Path., 31: 167, 1955.

16. Bloodworth, J.M.B. Jr., and Hamwi, G.J.
Experimental diabetic glomerulosclerosis.
Diabetes, 5: 37, 1956.

17. Collens, W.S., Banowitch, M.M. and Colsky, J.
Lipoprotein studies in diabetics with atherosclerotic disease.
J. Amer. Med. Ass., 155: 814, 1954.

18. Dolger, H.
Clinical evaluation of vascular damage in diabetes mellitus.
J. Amer. Med. Ass., 134: 1289, 1947.

19. Dolger, H.
Vascular complications of diabetes mellitus.
Bull. New York Acad. Med., 26: 779, 1950.

20. Dunlop, D.M.
Are diabetic degenerative complications preventable?
Brit. Med. J., 2: 383, 1954.

21. El-Mahallawy, M.N., Sabour, M.S., Osman, L.M. and Sadek, S.H.
Metaholic and endocrine aspects of diabetic nephropathy.
Brit. Med. J., 1, 674, 1960.

22. Engelberg, H., Goffman, T. and Jones, H.
Serum lipids and lipoproteins in diabetic glomerulosclerosis.
Diabetes, 1: 425, 1952.

23. Engelberg, H., Goffman, J. and Jones, H.
Serum lipids and lipoproteins in diabetic glomerulosclerosis.
Metabolism, 1: 300, 1952.

24. Farquhar, M.G., Hopper, J. Jr., and Moon, H.D.
Diabetic glomerulosclerosis: Electron and light microscopic studies.
Amer. J. Path., 35: 721, 1959.

25. Field, J.B., Federman, D.D., McDaniel, E. and Bakerman, H.
Urinary excretion patterns of some B-vitamins in diabetes: a study in patients with and without degenerative complications.
Diabetes, 6: 508, 1957.

26. Friedenwald, J.S.
Diabetic retinopathy.
Amer. J. Ophthal., 33: 1187, 1950.

27. Germuth, F.G. Jr.,
The mechanism of action of cortisone in experimental hypersensitivity II: Hypersensitivity of the serum sickness type.
J. Exper. Med., 98: 1, 1953.

28. Goetz, F.C., Hartman, J.F. and Lazarow, A.
Electron microscopy of the human glomerulus in early diabetes.
J. Clin. Investigation, 39: 991, 1960.

29. Hall, G.F.M.
The significance of atheroma of the renal arteries in K.W. Syndrome.
J. Path. Bact., 64: 103, 1952.

30. Hardin, R.C., Jackson, R.L., Johnston, T.L. and Kelly, H.G.
Development of diabetic retinopathy: Effects of duration and control of diabetes.
Diabetes, 5: 397, 1956.

31. Hartroft, W.S.
Fat emboli in glomerular capillaries of choline deficient rats and of patients with diabetic glomerulosclerosis.
Amer. J. Path., 31: 381, 1955.

32. Keiding, N.R., Mann, G.V., Root, H.F., Lawry, E.Y. and Marble, A.
Serum lipoproteins and cholesterol levels in normal subjects and in young patients with diabetes in relation to vascular complications.
Diabetes, 1: 434, 1952.

33. Kimmelstiel, R. and Wilson, C.
Intercapillary lesions in the glomeruli of the kidney.
Amer. J. Path., 12: 83, 1936.

34. Koss, L.G.
Hyaline material with reactions of fibrinoid in renal lesions in diabetes mellitus.
Arch. Path., 54: 528, 1952.

35. Larsson, Y., Lichtenstein, A. and Ploman, K.G.
Degenerative vascular complications in juvenile diabetes mellitus treated with "free diet".
Diabetes, 1: 449, 1952.

36. McLean, C.R., Hamilton, J.D. and Fitzgerald, J.D.L.
The effect of cortisone on experimental glomerulonephritis (Abstract).
Amer. J. Path., 27: 711, 1951.

37. McManus, J.F.A.
Medical Diseases of the Kidney.
LONDON, Lea and Febiger, 1948.

38. McNeal, P.S. and Rogers, J.
Complications of diabetes mellitus in Symposium on Diabetes and Obesity.
Med. Clin. North America, 39: 1607, 1955.

39. Mann, G.V., Gardener, C. and Root, H.F.
Clinical manifestations of intercapillary glomerulosclerosis in diabetes mellitus.
Amer. J. Med., 7: 3, 1949.

40. Murakami, R.
Beitrag zur Kenntnis der Veranderung des Neirenkorperchens beim diabetes mellitus.
Trans. Soc. path. jap., 26: 657, 1936.

41. Oliver, J., MacDowell, M. and Lee, Y.C.
Cellular mechanisms of protein metabolism in the nephron.
I: The structural aspects of proteinuria; tubular absorption, droplet formation and the disposal of proteins.
J. Exp. Med., 99: 589, 1954.

42. Rhodin, J.
Anatomy of kidney tubules.
International Review of Cytology VII, 1958, p. 485-534.
Editors: Bourne, G.H. and Danielli, J.F. Academic Press Inc. Publishers, NEW YORK, 1958.

43. Rich, A.R., Berthrong, M. and Bennett, I.L. Jr.,
The effect of cortisone upon the experimental cardiovascular-renal lesions produced by anaphylactic hypersensitivity.
Bull. Johns Hopk. Hosp., 87: 549, 1950.

44. Sabour, M.S., El-Mahallawy, M. and Abou-El-Nage, I.
The renal complications of diabetes mellitus: needle biopsy study of the renal histopathology.
Scott. Med. J., 6: 495, 1961.

45. Spühler, O. and Zollinger, H.V.
Die diabetische glomerulosklerose.
Schweiz. Med. Wschr., 73: 1430 and 1432, 1943.

46. Spühler, O. and Zollinger, H.V.
La glomerulosclerosis diabetica.
Sem. Med., (B. Aires), 2: 251, 1944.

47. Wernby, M.
La glomerulosclerose diabetique.
Rev. med. Suisse rom., 64: 615, 1944.

48. White, P.
Natural course and prognosis of juvenile diabetes.
Diabetes, 5: 397, 1956.

49. Wilens, S.L. and Stumpf, H.H.
Nodular and fatty glomerular lesions in rabbits on cortisone.
Amer. J. Path., 31: 275, 1955.

50. Wilson, J.L., Root, H.F. and Marble, A.
Prevention of degenerative vascular lesions in young patients by control of diabetes.
Amer. J. Med. Sc., 221: 479, 1951.

51. Zubrod, C.G., Eversole, S.L. and Dana, G.W.
Ameliocation of diabetes and striking rarity of acidosis in patients with Kimmelstiel-Wilson lesions.
New Englan. J. Med., 245: 518, 1951.

MICROANATOMY OF THE RENAL TUBULES.

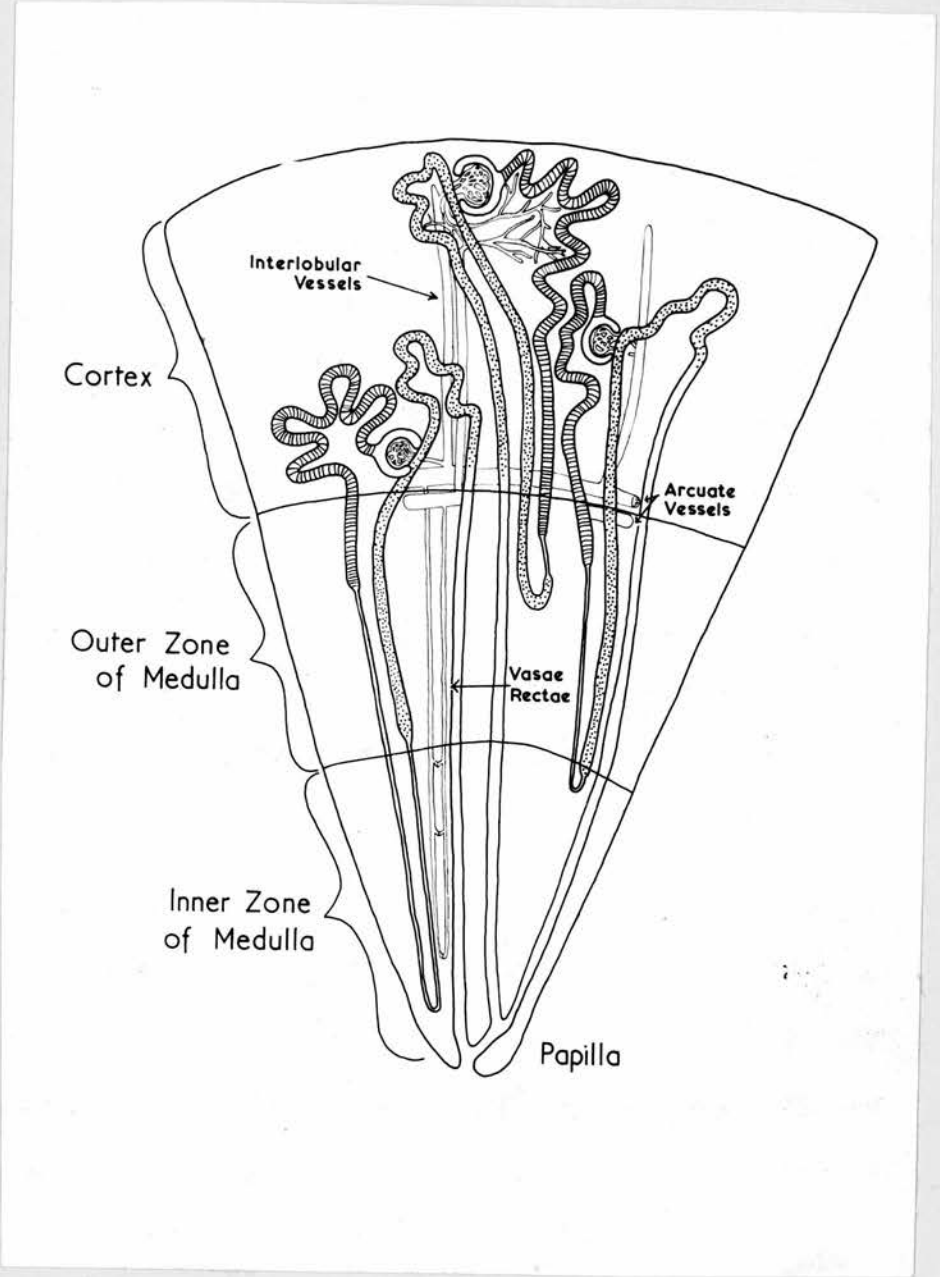

Interlobular Vessels
Cortex
Arcuate Vessels
Outer Zone of Medulla
Vasae Rectae
Inner Zone of Medulla
Papilla

Diagram 3.

MICROANATOMY OF THE RENAL TUBULES.

The object of this study is to analyse more closely the ways which the glomerular filtrate takes in its passage along the renal tubules and to examine the functional significance of these movements. For this, a morphological study of the minute structure of the tissue elements which the glomerular filtrate has to traverse before it appears as the final urine, is needed. The microanatomy of the renal tubules has been reviewed in the "General Introduction". In the following study, I was not so much concerned with a meticulous naming and description of all the tissue and cell structures present, as with emphasising a few findings which appear essential for an understanding of the physiological considerations.

Tubules from three mammalian species were studied by the electron microscope, man, rabbit and "wistar" and "Sprague-Dawley" rats. Realising the fact that there is at least two types of nephrons, the superficial cortical nephrons and the juxtamedullary nephrons in these mammalian species, particular care was taken in choosing the blocks from the experimental animal's kidneys. Blocks were taken from the superficial cortex where the kidney had a brown colour, from the corticomedullary region which was easy to identify on account of its reddish tint, and from the renal papilla which was pale in colour. The blocks from each zone were processed separately and were called "cortex", "outer medulla" and "inner medulla" respectively

The differences between the superficial cortical nephrons andthe juxta-medullary nephrons are quite significant (Diagram 3). The cortical nephrons have short loops which at best reach the outer zone of the medulla, while

many, perhaps most, turn in the cortex. In these, the thin segment of the loop of Henle is very short or entirely absent (9), and if present, extends for a short distance along the descending limb and does not go around the loop or extend into the ascending limb. The juxtamedullary nephrons have long loops that reach down to the tip of the papilla, and have an extended thin segment along the descending limb, the loop and for some distance along the ascending limb. However, at one particular level, all the ascending limbs of Henle's loops change from thin to thick segments and it is this level that subdivides the medulla into an outer and an inner zone. So any thin segment seen in a block from the outer medulla must be on the descending limb of the loop of Henle, while in the inner medulla it is not possible to ascertain whether a thin segment is ascending or descending. The nephrons in between those two extreme examples have loops that dip for a variable distance in the outer medullary zone and though the thin segment might extend to the bend of the loop, it does not extend along the ascending limb. The juxtamedullary nephrons also differ from the rest in their blood supply. Their efferent glomerular arteriole has the same size as the afferent and dips down into the medulla to form capillary loops around the loops of Henle, the "vasae rectae" instead of branching around the convoluted tubules in the cortex. These facts which have been described more than half a century ago (9) are not generally realised. Only about one in seven nephrons in the human kidney have long loops (6) and at least 70% of the nephrons in the rat kidney have short loops (15). These observations have been clearly substantiated in recent measurements reported by O'Dell and B. Schmidt-Nielsen (7).

The Proximal Tubule.

The proximal tubule is divided in two parts: the pars convoluta, in the cortex, and the pars recta, in the outer medulla (Diagram 3). The pars recta of the proximal tubule constitutes the beginning of the descending limb of the loop of Henle and is about $\frac{1}{3}$ of the whole proximal tubule.

In the pars convoluta, the luminal cell surface has a broadened fairly regular brush border (Fig. 121 and 122). This consists of microvilli which are so densely packed together that in transverse sections a honeycomb pattern is observed (Fig. 123). In freshly fixed material, it is usually impossible to see any free tubular lumen (Fig. 124). Directly below the brush border (Fig. 121 and 122) there is a narrow zone of cytoplasm with small rounded or tubular spaces, some of which are connected with the spaces between the microvilli and thus with the lumen of the tubule. Other vacuoles are situated at a deeper level and do not show any such communication with the lumen - at least not in the same plane of section. Sometimes, elements of the endoplasmic reticulum and few mitochondria can be seen in this relatively clear apical part of the cell. The basal part of the cell, which occupies more than half the height of the cell, contains the nucleus. Numerous regular deep folds of the basal cell membrane can be seen to extend as far as the mid-level of the cell or even further. These folds split up the basal half of the cell into numerous cytoplasmic layers in a lamellar arrangement, in which the greater part of the mitochondria are to be found, in very close contact with the cell membrane (Fig. 125). The intervals between these cytoplasmic layers, being due to plication of the membrane, are closed towards the cell and open towards the basement membrane. It is quite clear that these interspaces are in fact extracellular and form a

complicated system of narrow slits dividing the basal half of the cell forming a sort of a "basal labyrinth".

The cells of the pars recta of the proximal tubule show some definite morphological differences from those of the pars convoluta; however, the transition from one cell type to the other occurs gradually. The cells (Fig. 126 and 127) gradually get shorter, from a height of about 8 μ in the pars convoluta to a height of about 5 μ towards the thin segment of the loop of Henle. In most cases a definite lumen is present. The microvilli are loosely packed, less orderly and are relatively short and plump. The cytoplasm is less dense than in the pars convoluta and the vesicles and tubular invaginations are more numerous (Fig. 126). The mitochondria are smaller, more rounded, less numerous and are distributed fairly evenly throughout the cell, as is the endoplasmic reticulum. The basal cell foldings decrease both in number and height from the neck of the nephron downwards, and in the pars recta they are short and do not play any real part in the cytoarchitecture, especially in the arrangement of the mitochondria. One more feature worth mentioning is the frequent finding of densely grouped vesicles often arranged in rows in the basal cytoplasm directly above the basal cell membrane, as well as the presence of numerous large osmiophilic granules of finely granular and lamellated material.

The Loop of Henle.

Three different types of epithelium line the loop of Henle; thick descending part, thin part and thick ascending part. The thick descending part is actually the pars recta of the proximal tubule, while the thick

segment of the ascending limb is the pars recta of the distal tubule.

In contrast to the highly differentiated epithelium of the thick parts, the thin segment, in both its descending and ascending parts, has an epithelium composed of flat cells of very simple structure, circumscribing a wide lumen (Fig. 128). The luminal cell membrane has few short microvilli. The cytoplasmic matrix is fairly clear and contains only a few RNP granules as well as small rounded vesicles and relatively wide cisterns of the endoplasmic reticulum. The mitochondria are very small and are sparsely distributed in the basal part of the cell in a quite unsystematic pattern. There are a few shallow plications of the cell membrane and quite often interdigitations with neighbouring cells; the interdigitating lamellae are sealed off from the tubular lumen by terminal bars, in the same way as adjacent cells are sealed off from the lumen (Fig. 129 and 130).

The Distal Tubule.

The distal tubule consists of three parts; the pars recta or the thick segment of the ascending limb of the loop of Henle, and this is present in the outer medulla; the macula densa (which has already been described); and the pars convoluta, in the cortex. On the whole, the cells of the distal tubule have the same general structure, with some differences in the different parts.

The epithelium of the distal tubule distinctly resembles that of the proximal tubule in its "polar" architecture and in its fine structure, but differs from it in certain essential points.

The distal tubule always has a patent lumen (Fig. 131). The luminal surface has no brush border, but only occasional short microvilli. The

apical cytoplasmic zone usually shows less numerous vesicles, and the nuclei of the cells are not situated in the basal zone, but predominantly towards the apex of the cell. All the other cell structures are practically indistinguishable from those of the proximal tubule, particularly with regard to the density of the cytoplasm as a result of the high content of RNP granuoles - and the characteristic structure of the basal half of the cells with the plication of the cell membrane. These cells also show a definite "basal labyrinth" which resembles that of the proximal tubules in every detail (Fig. 132).

In the first part of the thick segment of the ascending limb of the loop of Henle, in the outer medulla, the cells are cuboidal, have a rather clear cytoplasm and the mitochondria are oval in shape and not particularly basal in position (Fig. 133). However, as the cortex is approached, the cells become more columnar, the cytoplasm darkens, and the mitochondria increase in number and length (Fig. 134). The long, slender mitochondria, arranged longitudinally in the cytoplasmic lamellae within the basal cell plications, are particularly typical of the pars convoluta of the distal tubule (Fig. 132).

The Collecting Tubules.

The collecting tubules have a relatively wide lumen and are lined by a low cubical epithelium which is different from that of the preceeding segments (Fig. 135). The cells have few short microvilli, interdigitate with one another (Fig. 136) and have few, shallow plications of their basal cell membrane. The most striking feature is the light appearance of the cytoplasm. This is due partly to the low density of the cytoplasmic matrix itself, and secondly to the scarcity of the cytoplasmic organelles, which

are loosely scattered without any apparent pattern. The mitochondria are remarkably small. The nucleoplasm gives a strikingly loosely knit impression, in the same degree as the cytoplasm appears transparent. All these characteristics become increasingly marked as one progresses distally, so that the collecting tubule at the tip of the papilla consists of very transparent cells, which are relatively lacking in structure, especially in mitochondria and do not interdigitate with one another but are merely in contact. (Fig. 137). The adjacent tubular cells are sealed off on the luminal sides by terminal bars.

This is the description of what has been termed the "light" cell. In addition, there is another type of cell, the so-called "dark" cell. This "dark" cell (Fig. 138) has been found all along the collecting tubules whether they were"cortical", "outer medullary" or "inner medullary" collecting tubules, but they only appeared regularly in the "cortical" and "outer medullary" collecting tubules, were quite rare in the "inner medullary" zone and were entirely absent at the tip of the papilla. Some appeared as if "inserted" between the "light cells" or "intercalated" between them and the tubular basement membrane (Fig. 139), giving the impression that the cell has been wedged in. These cells appear dark because of a large number of foldings of the basal basal cell membrane, a large number of RNP granules and larger and more numerous mitochondria (Fig. 138). They have much more microvilli than the "light" cells and the cytoplasm has many more vesicles.

However, by examining a large number of collecting tubules, a continuous array of cells could be found, ranging from light cells with few vesicles and microvilli (Fig. 137) through light cells with many vesicles and microvilli, and basal cell foldings (Fig. 140) to dark cells with many vesicles

and microvilli (Fig. 138). Also the cells of the so-called "arched" or "connecting portion" of the collecting tubule, which connects the distal tubule with the collecting duct conform to the classical description of the "dark cells" and I think that there is a sequence of transitional morphological forms according to the position of the cell along the collecting tubule, with a considerable degree of overlapping between the cells of one type and that of the preceeding and the next in any given segment of the collecting tubule.

The General Architecture and the Capillary System of the Kidney.

The general arrangement of the tubules within the ground substance of the kidney and their spatial relationship with the capillary system, has been over-looked by most previous authors. A definite architectural pattern was found in this study for the renal tubules and the renal capillaries which differs in the three zones of the kidney.

In the Cortex.

The relationship between the epithelium of the convoluted tubules and the endothelium of the peritubular capillaries is as close and intricate as that in the glomerulus. Here too, a common basement membrane is shared between the epithelial and endothelial cells (Fig. 141 and 142). The tubular basement membrane under the electron microscope has the same appearance as that of the glomerulus. It is separated from the plicated basal cell membrane by a narrow clear cleft which is continuous with the spaces of the "basal labyrinth" described above. In other words, the "extracellular" space of the basal labyrinth is closed on the cellular side by the cell membrane and on the capillary side by the basement membrane (Fig. 132)

The capillary endothelial cells, which usually lie in the corners between the tubules, extend into very flat cytoplasmic processes, which almost completely cover the irregularly shaped spaces between the tubules, like a carpet (Fig. 143). This endothelial "carpet" moreor less follows the contours of the tubules, which,one might say, lie in a blood lake (Fig. 143). The attenuated endothelial cytoplasm of the peritubular capillaries is fenestrated, just as that of the glomerular capillaries. However, the fenestrae are smaller (150 - 500 Å in diameter), and are more frequent than in the glomerular capillaries. The blood plasma is thus indirect contact with the basement membrane here also.

Interstitial cells are not infrequently seen between the tubules, and may be closely surrounded by fibrils, but the interstitial space is very narrow.

In the Outer Zone of the Medulla.

The renal medulla is very well supplied by capillaries. In the outer medullary zone the tubules lie closely packed together with few peritubular capillaries with which they share a common basement membrane (Fig. 144). This sharing of a common basement membrane between a capillary and an adjacent tubule was constantly observed for the pars recta of the proximal tubule and for the thin and thick segments of the loop of Henle, but was hardly ever seen with collecting tubules (Fig. 145). In general each tubule is in contact with several capillaries and over a considerable portion of its length. These capillaries have an attenuated fenestrated endothelial lining (Fig. 146) like the glomerular and the peritubular cortical capillaries.

Apart from the tubules, a large tuft of vasae rectae can be seen, well away from the collecting tubules and the thick segments of Henle's loops,

but in close proximity to the thin segments (Fig. 147). All the capillaries and the tubules are closely packed together with little interstitial space.

Two different types of capillary can be seen in the outer zone of the medulla, particularly in the tuft of vasae rectae. Onehas an attenuated fenestrated lining (Fig. 148) while the endothelium of the other type is thick, non fenestrated (Fig. 149) and frequently shows multiple branching processes (Fig. 150). The pattern of their distribution is remarkable. Both types frequently abut one on the other over a great distance of their circumference, and for quite a good length, separated only by their basement membranes and a very narrow interstitial space. Sometimes, they share a common basement membrane for some length (Fig. 151). Two similar types of capillary rarely run close together, and if they do they never share a common basement membrane. The renal tubules are related chiefly to the fenestrated type of capillary and scarcely at all to the other variety, and they never share a common basement membrane with the thick type of capillary (compare Fig. 146 with Fig. 152).

The thick type of capillary is lined by an endothelium ranging in height from 0.2 - 5.0 μ ; the cells of which frequently overlap broadly (Fig. 149). The free edges of the underlying cells are frequently dentate or fusiform, and when cut across can be seen as small rounded bodies between an overlying endothelial cell and the capillary basement membrane (Fig. 150 and 151). The cytoplasm of these endothelial cells contains moderate numbers of small mitochondria and vesicles (Fig. 149), indicating that they perform a pinocytotic function. The free surface of the cytoplasm frequently extends microvilli into the capillary lumen (Fig. 150). The basement membrane

surrounds the vessel as a whole. Outside the basement membrane, pericytes are sometimes seen closely applied to the vessel (Fig. 153).

The thin type of capillary stands in strong contrast to the thick type just described. Although the endothelial cells may have substantial thickness, they characteristically show the tenuous fenestrated appearance seen in the glomerular and cortical capillaries (Fig. 148). The cytoplasm is frequently interrupted entirely, and the continuity of the cell is maintained only by the fused membranes from the two sides of the cell which frequently show pores. The interruptions of the cytoplasm are sufficiently regular so that in section the endothelium frequently has a beaded appearance.

In the Inner Zone of the Medulla.

The inner medulla contains only thin loops, collecting tubules and capillary blood vessels. The renal tubules and the capillaries are diffusely scattered, without any recognisable pattern, in a wide space of ground substance which appears almost homogeneous even under the electron microscope (Fig. 154). The tubules and capillaries have their own separate basement membrane and are not in contact.

As a result of this apparently "independent" character of the capillaries in the inner medulla, relatively wide interstices are seen between the capillaries and the urinary tubules, in which interstitial cells are found. The interstitial cells are rather large cells with a round or oval nucleus and a cytoplasm that contains few mitochondria and vacuoles (Fig. 155). The cytoplasm is divided into several branches which are frequently seen to be in contact with the renal tubules and capillaries showing a common basement membrane within them (Fig. 156). The most striking features of these cells is their very high content of very densely osmiophilic granuoles, identical

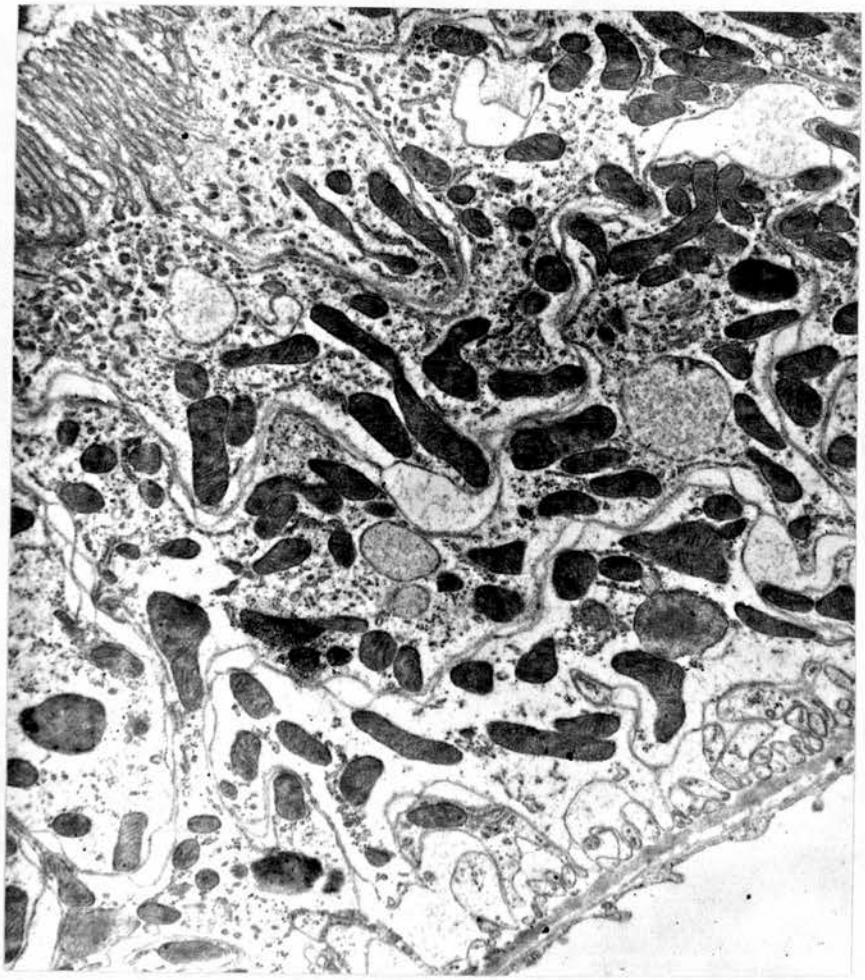


Fig. 121. Proximal convoluted tubule from an albino rat. The luminal surface is covered by microvilli; the space underneath is relatively free of mitochondria but contains a large number of microbodies and RNP granules. Vacuoles with clear and granular contents are seen throughout the cytoplasm. Some of them are obviously continuous with the lumen through a narrow tubular slit. The vacuoles lie between two membranes of the endoplasmic reticulum, and this double membrane after pursuing a sinuous course in the cytoplasm, interrupted by cystic dilatations, eventually reaches the basement membrane. Note the common B.M. of the cell and the adjoining capillary. x 15,000

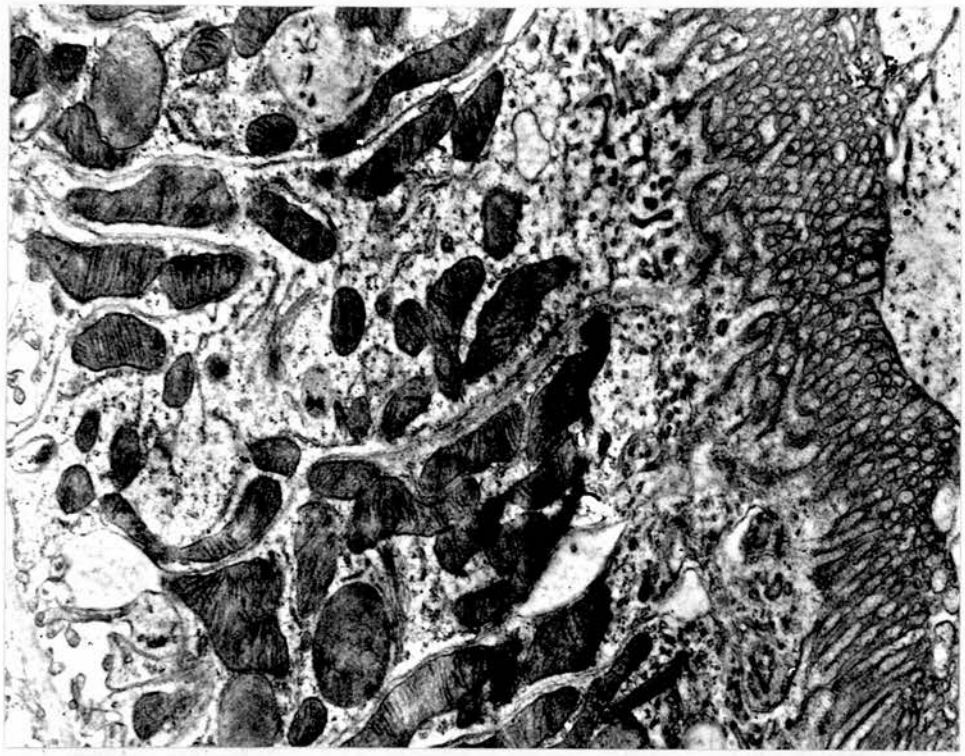


Fig. 122. Proximal convoluted tubule from a normal rabbit. The luminal surface has a brush border consisting of microvilli beneath which is a narrow zone of cytoplasm free from mitochondria but contains microbodies, RNP granuoles and rounded and tubular vacuolar spaces. x 15,000

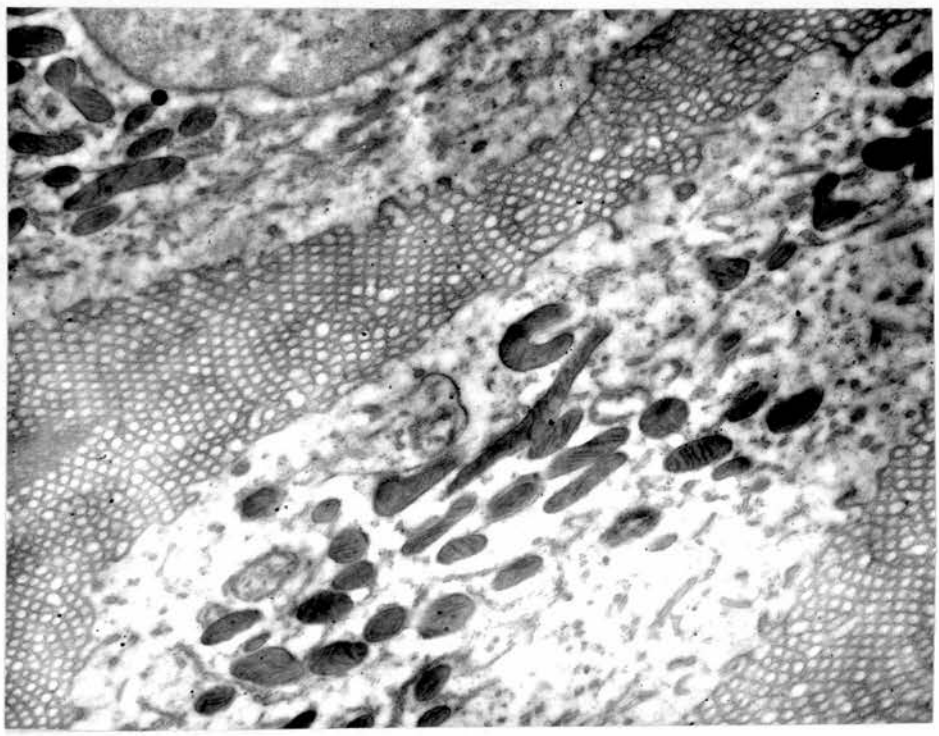


Fig. 123. Proximal convoluted tubule from an albino rat. The microvilli are densely packed together giving a honeycomb appearance with no real lumen. x 20,000

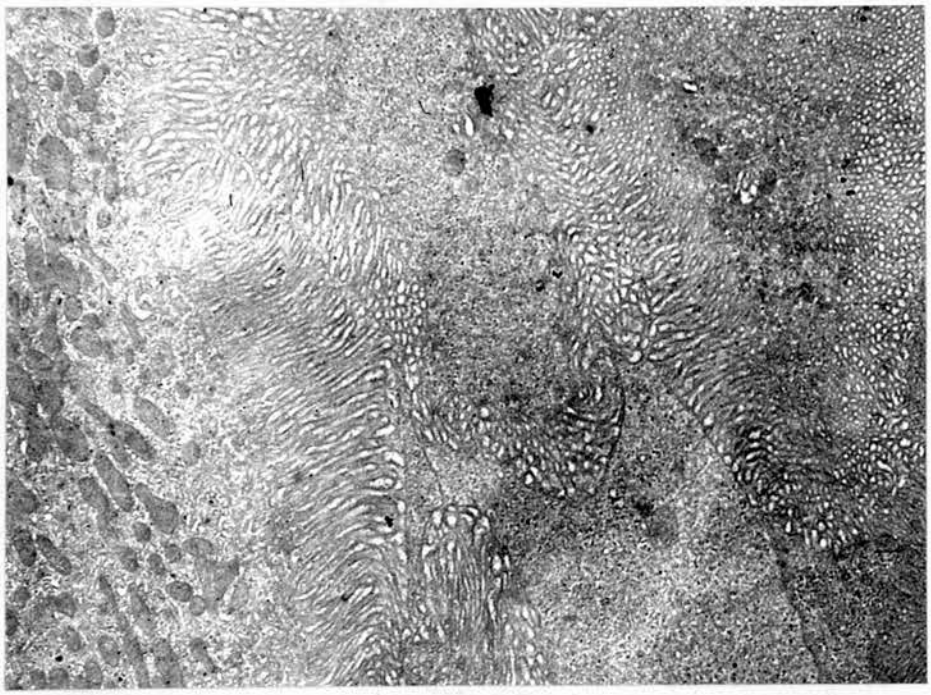


Fig. 124. Proximal convoluted tubule from a hooded rat. Note that the microvilli are so closely packed that it is impossible to see any free lumen. x 6,000

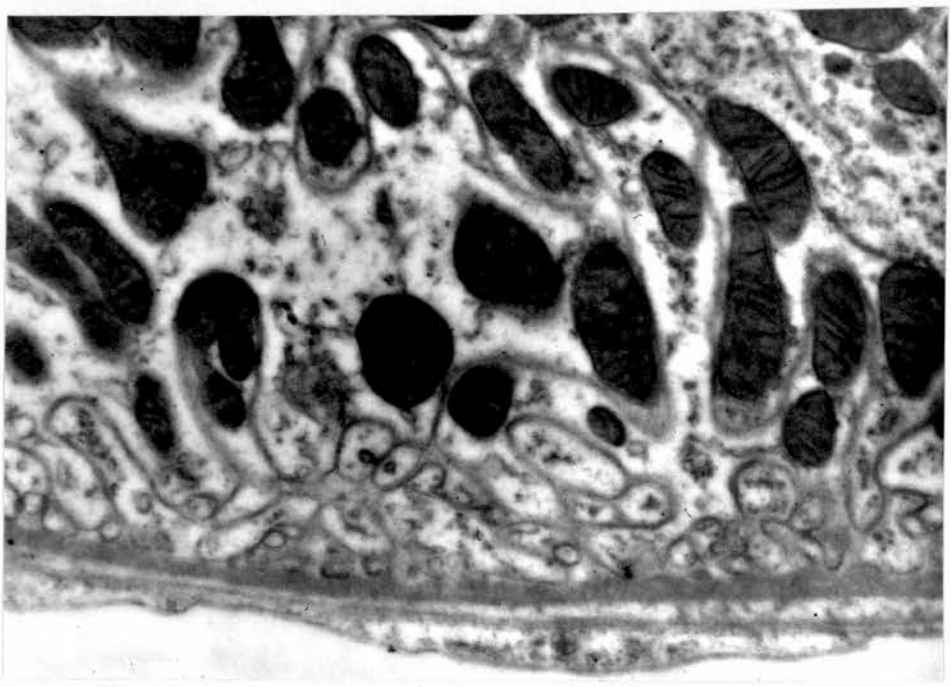


Fig. 125. The basal part of a proximal convoluted tubule from an albino rat. Note how the basal cell foldings split up the cytoplasm in a lamellar fashion as well as their close proximity to the mitochondria. x 24,000

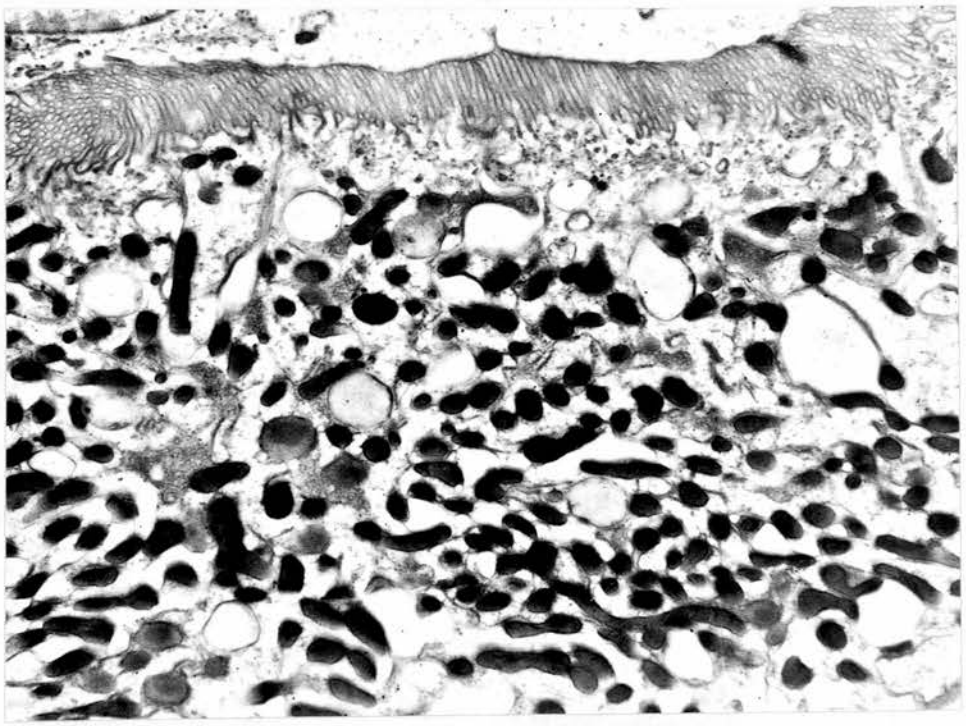


Fig. 126. A cell from the pars recta of a proximal tubule of an albino rat. Note that the cell is shorter and the microvilli are shorter than in the pars convoluta. A definite lumen can be seen at the top. The cytoplasm is less dense and contains more vacuoles than in the pars convoluta. x 6,000

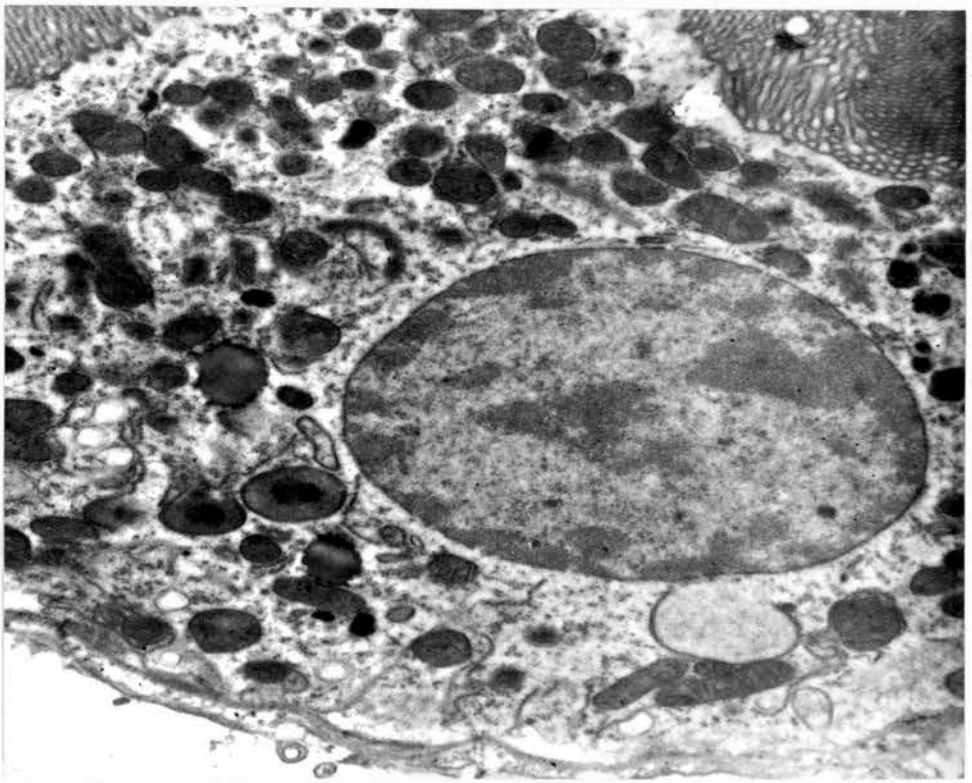


Fig. 127. A cell from the pars recta of a proximal tubule from an albino rat. Note that the basal cell foldings are very scanty. The mitochondria which are distributed rather evenly throughout the cell are smaller and more rounded than in the pars convoluta. x 12,000.

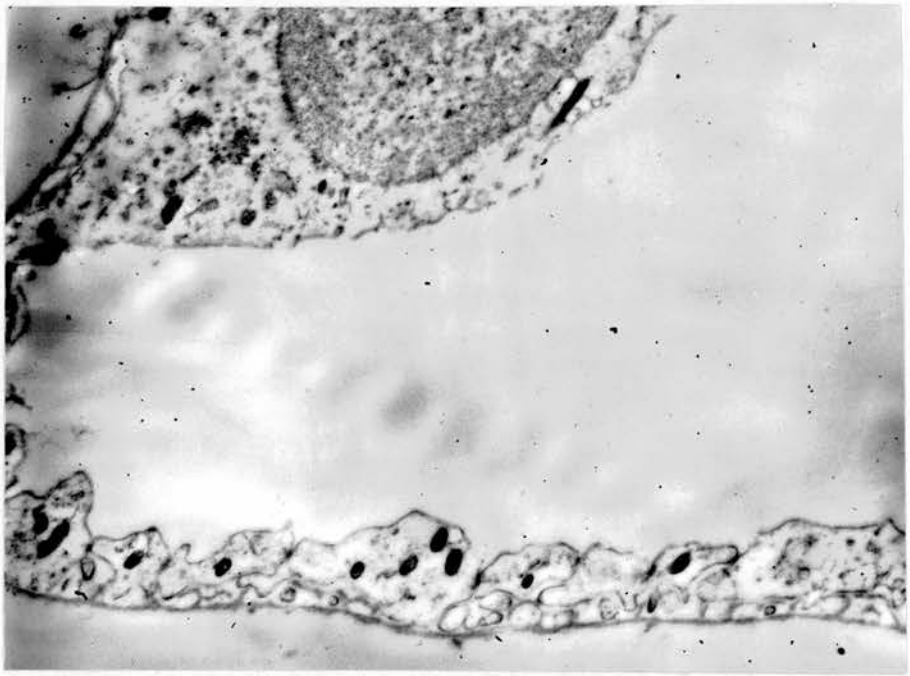


Fig. 130. Thin segment of the loop of Henle from an albino rat. Note the intercalated lamellae and the terminal bars. x 12,000

Fig. 131. Distal tubule from an albino rat. A definite lumen can be seen and no microvilli cover the luminal surface of the cells. Note that the mitochondria are massed in the basal part of the cells and that the tubule shares a common basement membrane with the adjacent capillary. x 2,500

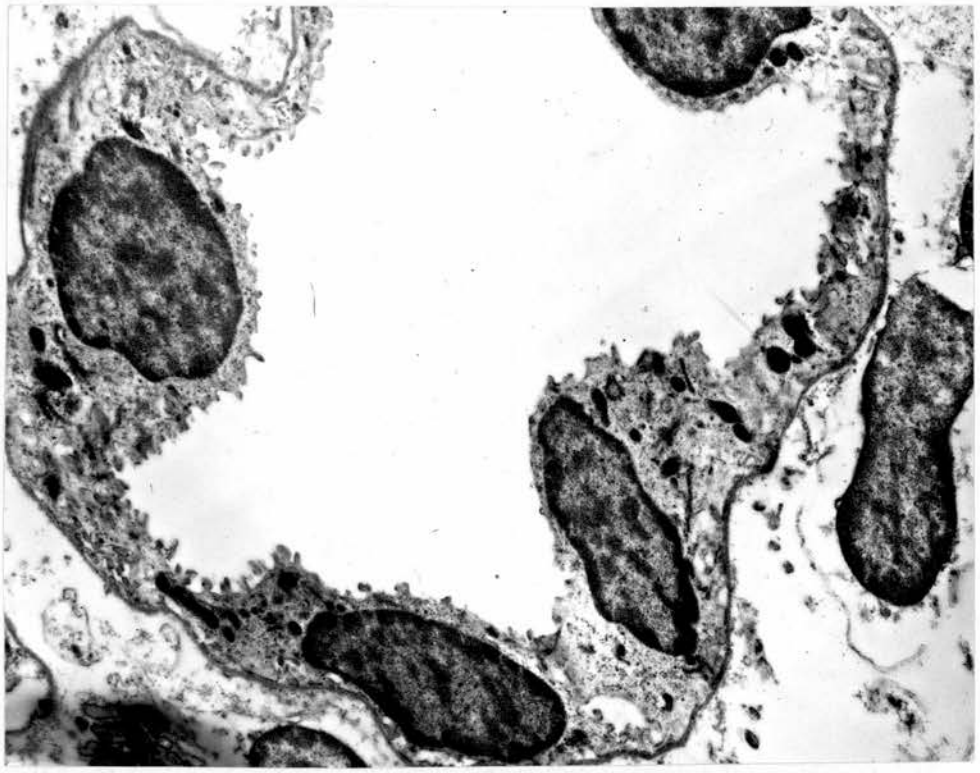


Fig. 128. Thin segment of a loop of Henle from a normal albino rat. x 6,000

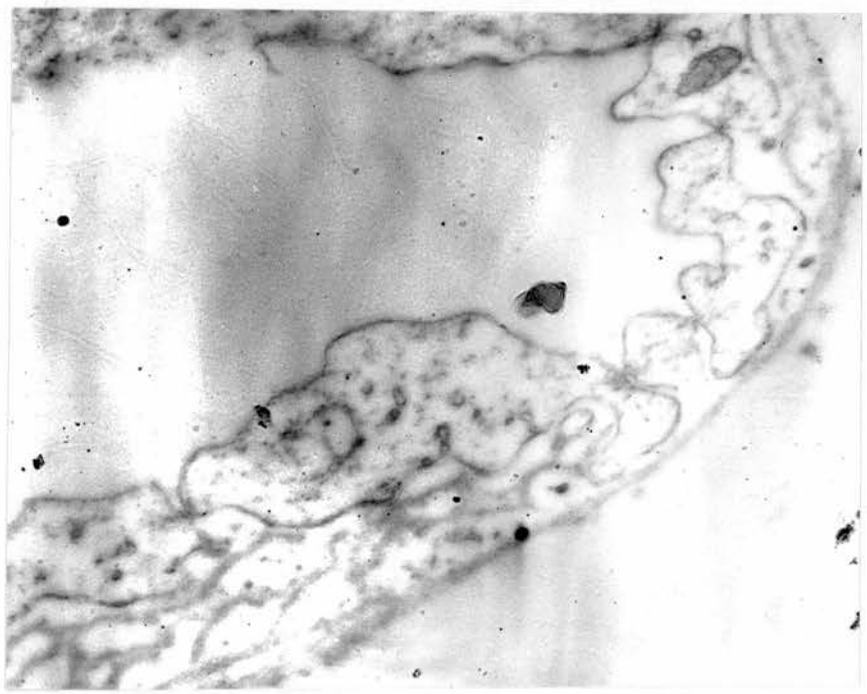


Fig. 129 Thin segment of a loop of Henle from an albino rat. Note the terminal bars sealing off the spaces in between the interdigitating lamellae from the tubular lumen. x 12,000

Fig. 132. The basal part of a distal convoluted tubule from an albino rat. Note the plication of the basal cell membrane with the long, slender, sausage-shaped mitochondria sandwiched in the cytoplasmic lamellae created by this plication. The basal labyrinth is beautifully demonstrated in this electron micrograph. x 15,000

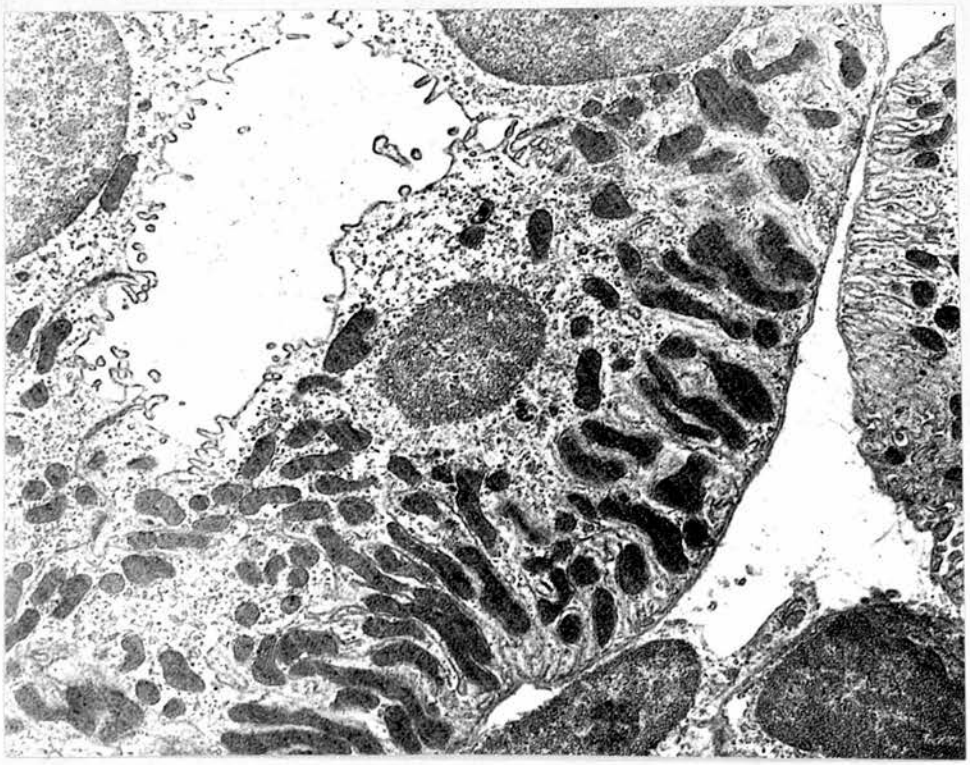


Fig. 133. The first part of the thick segment of the ascending limb of the loop of Henle, in the outer medullary zone, close to the thin segment, from a hooded rat. The cells are cuboidal, the mitochondria are shorter than in the pars convoluta, and are not particularly basal in position. x 6,000

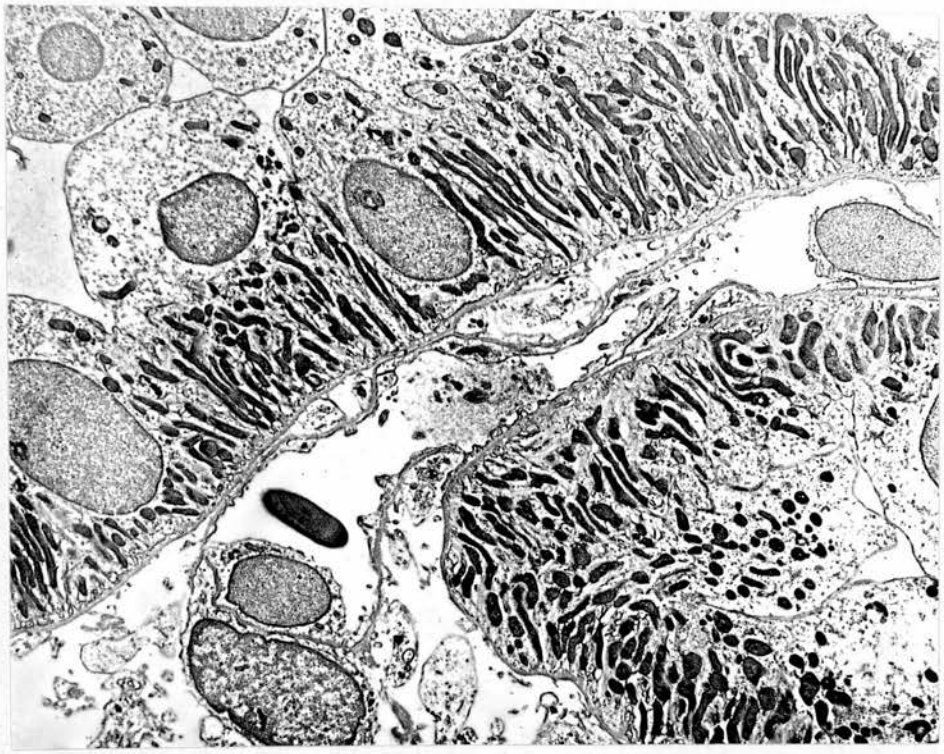


Fig. 134. Distal tubules from the cortico-medullary region of an albino rat. The cells are more columnar and the mitochondria more elongated than in Fig. 133, but not as long and slender as those in Fig. 132. x 2,500

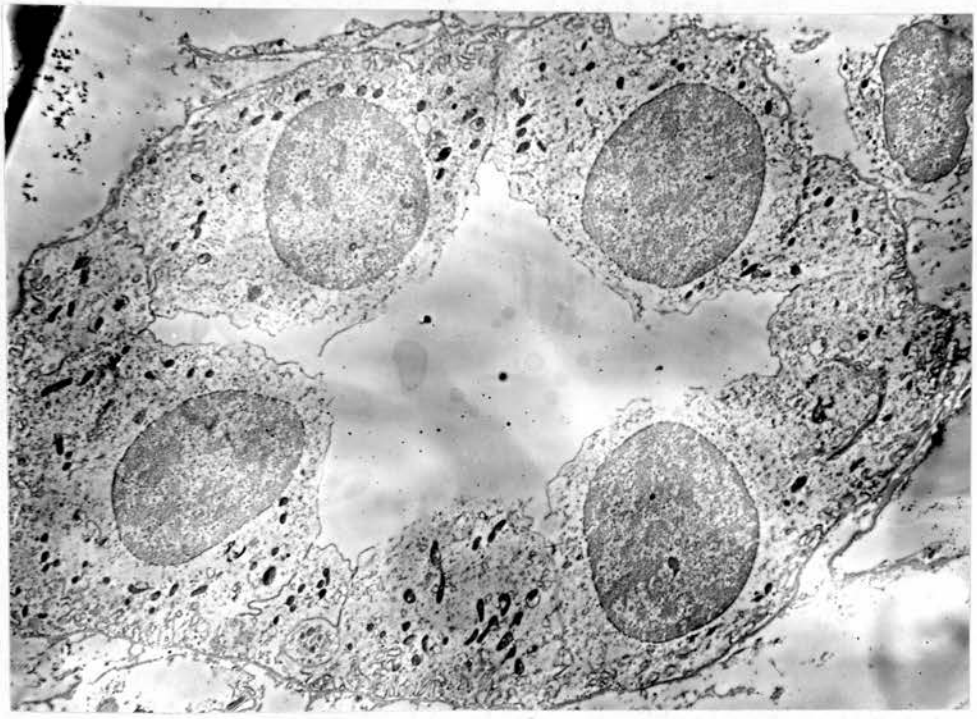


Fig. 135. A collecting tubule from the inner medulla of an albino rat. The lining cells are cubical and have a clear cytoplasm and few cell organelles. The lumen is relatively wide. x 2,500

Fig. 136. Part of a collecting tubule, from the inner zone of the medulla of an albino rat. Note the cytoplasmic process wedged in between two adjacent cells. x 9,000

Fig. 137. Cells of a collecting tubule in the papilla of an albino rat. Note the very clear appearance of the cytoplasm and the extreme poverty in cell organelles. The cells merely lie in contact with one another without any interdigitation. x 6,000

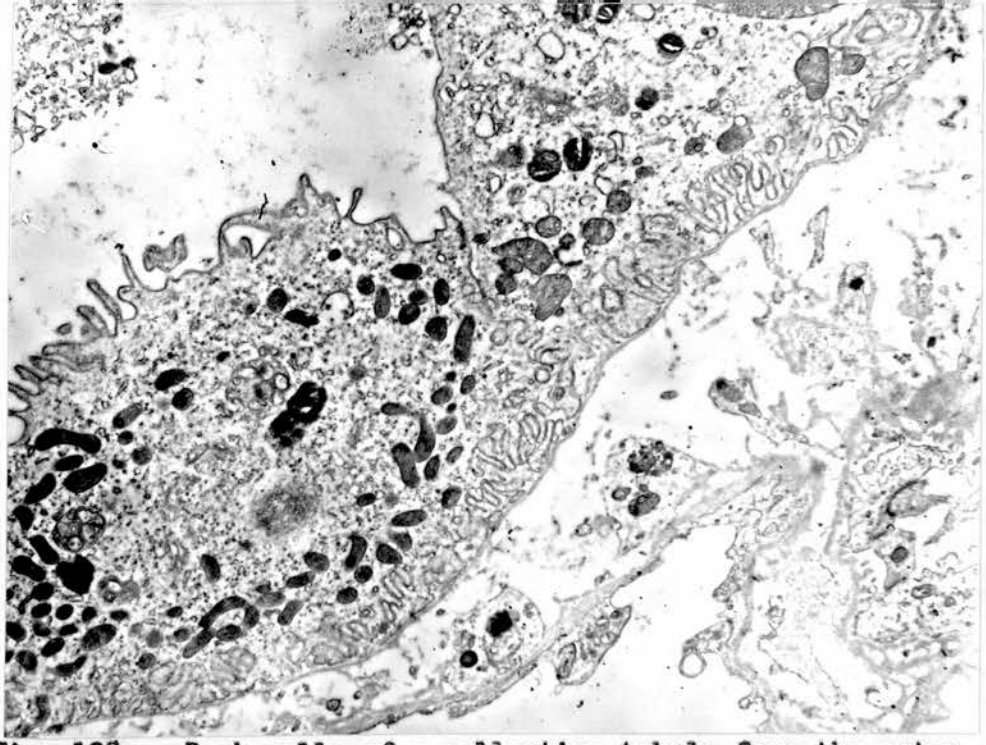


Fig. 138. Dark cells of a collecting tubule from the outer medulla of an albino rat. Compared with the light cells (Fig. 136 & 137) these cells have more mitochondria and more RNP granules in the cytoplasm. The luminal surface has a number of short microvilli and the basal cell surface is thrown into a number of shallow folds. x 6,000

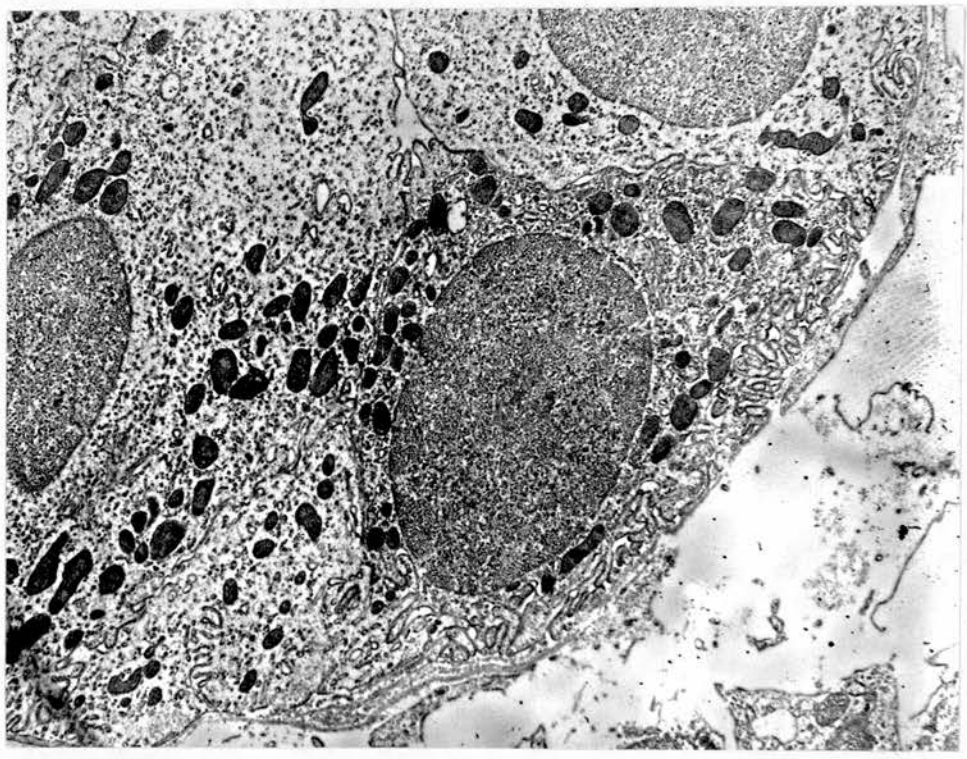


Fig. 139. A dark cell in a cortical collecting tubule of an albino rat wedged in between two light cells and intercalated between them and the tubul ar basement membrane. x 6,000

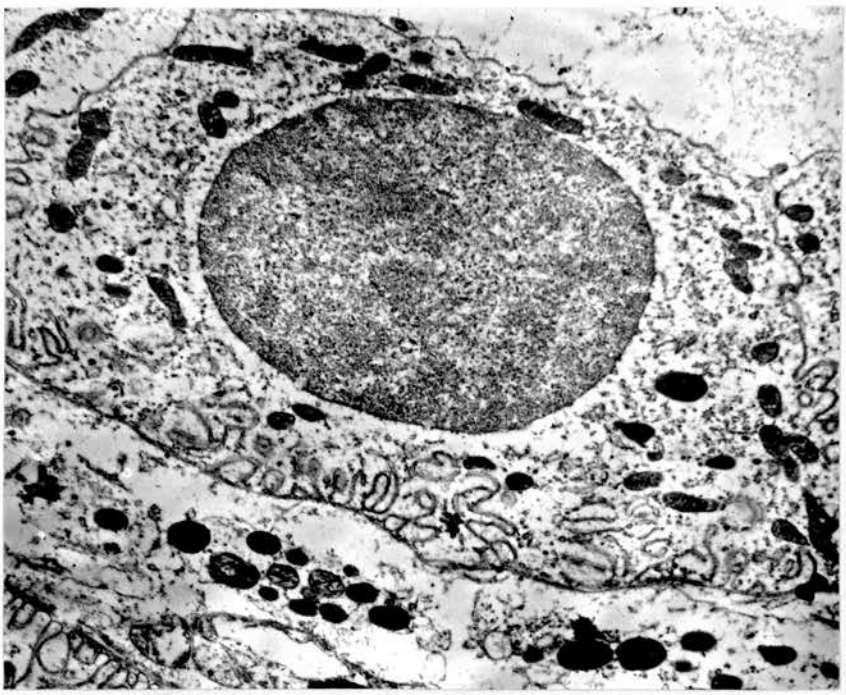


Fig. 140. A cell from an outer medullary collecting tubule of an albino rat. This cell is darker than the light cells seen in Fig. 137, and has more mitochondria and more basal cell foldings, but is lighter than the dark cells seen in (Fig. 138) and has fewer mitochondria and fewer microvilli. x 9,000

Fig. 141. The basal part of a proximal convoluted tubule from a hooded rat sharing its basement membrane with a capillary lined by attenuated fenestrated endothelium. x 15,000

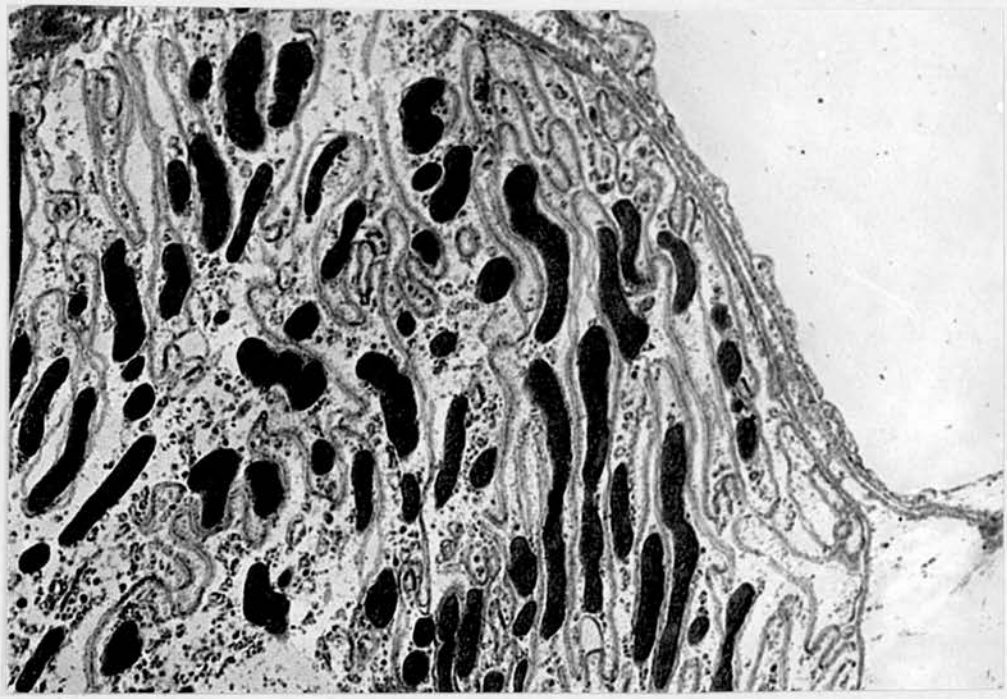


Fig. 142. The basal part of a distal convoluted tubule and an adjacent capillary from an albino rat. x 6,000

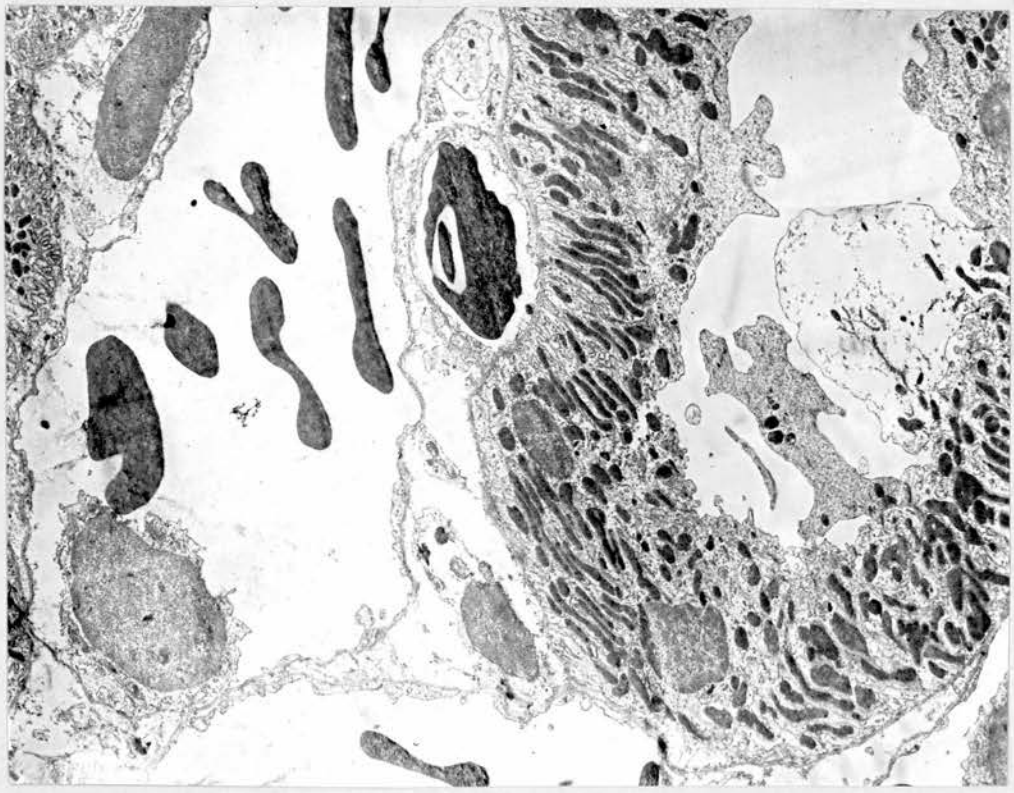


Fig. 143. Thick segment of the loop of Henle from an albino rat's kidney. Four capillaries are seen, sharing a common basement membrane with the tubule in part of their circumference and almost completely filling the intertubular space, so that the tubule is lying in a "blood lake".
x 4,000

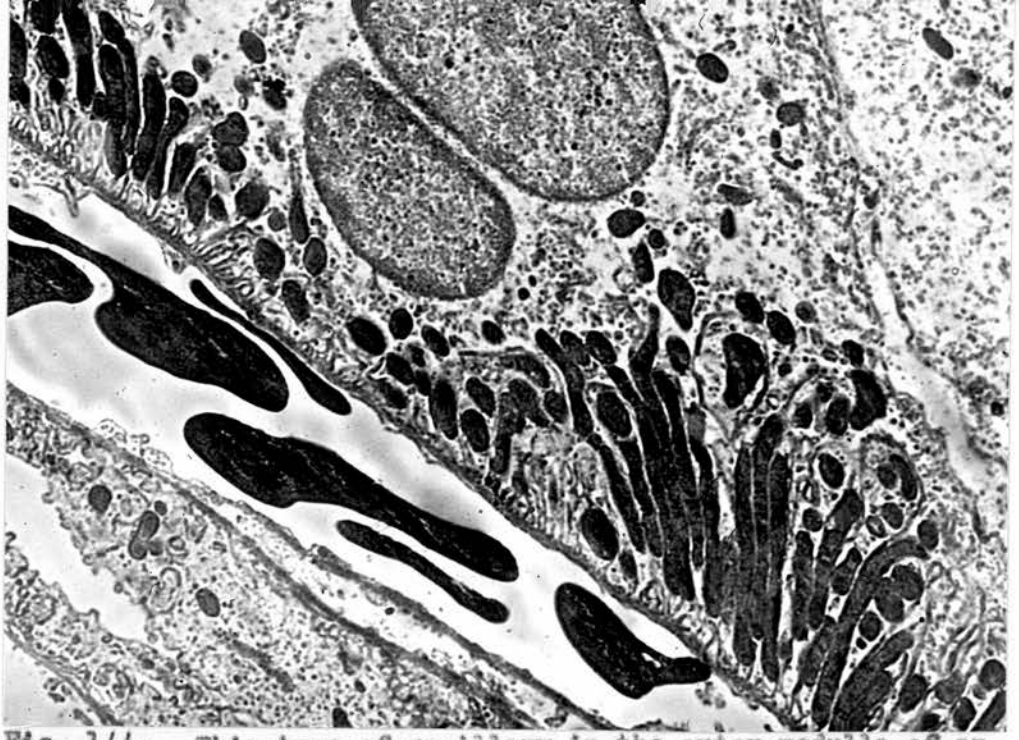


Fig. 144. Thin type of capillary in the outer medulla of an albino rat's kidney sharing its basement membrane with thick segment of the loop of Henle above, and with a thin segment below. x 5,000

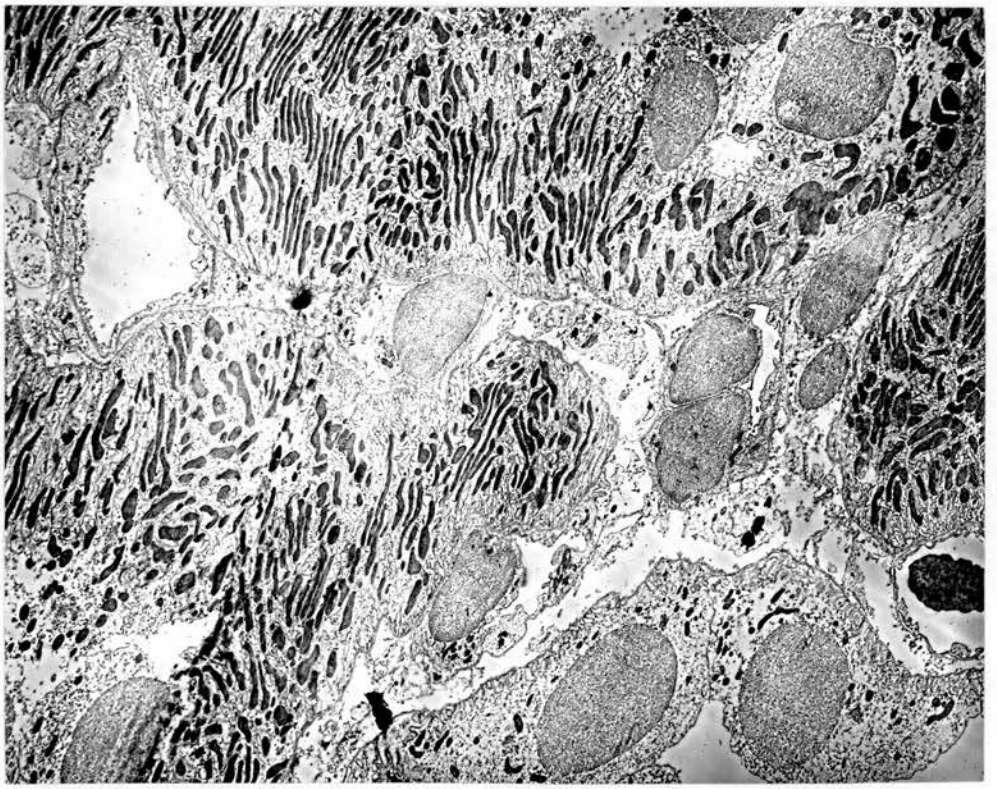


Fig. 145. A low power electron micrograph from an albino rat showing the pattern of arrangement and relationship between the tubules and the capillaries in the outer zone of the medulla. Five capillaries can be seen sharing their basement membrane along part of its circumference with that of adjacent thick segments. The collecting tubule, at the bottom is quite separate from the adjacent capillary. x 1000

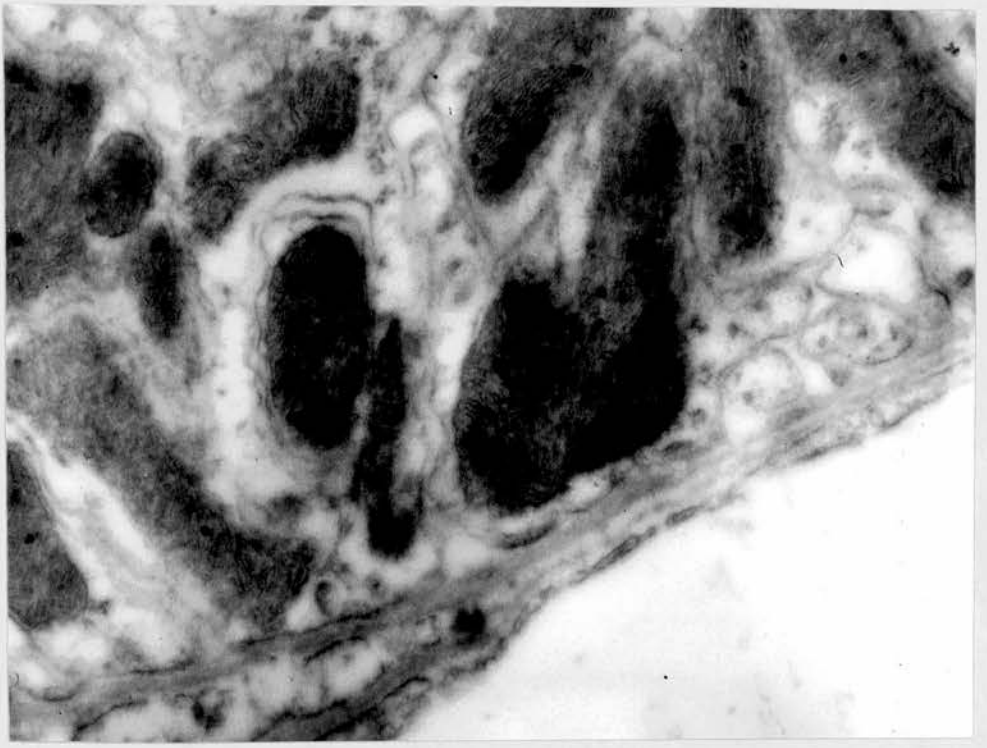


Fig. 146. Thick segment of the loop of Henle from an albino rat with its adjoining capillary. Note that the endothelium of the peritubular capillary is attenuated and fenestrated. x 45,000

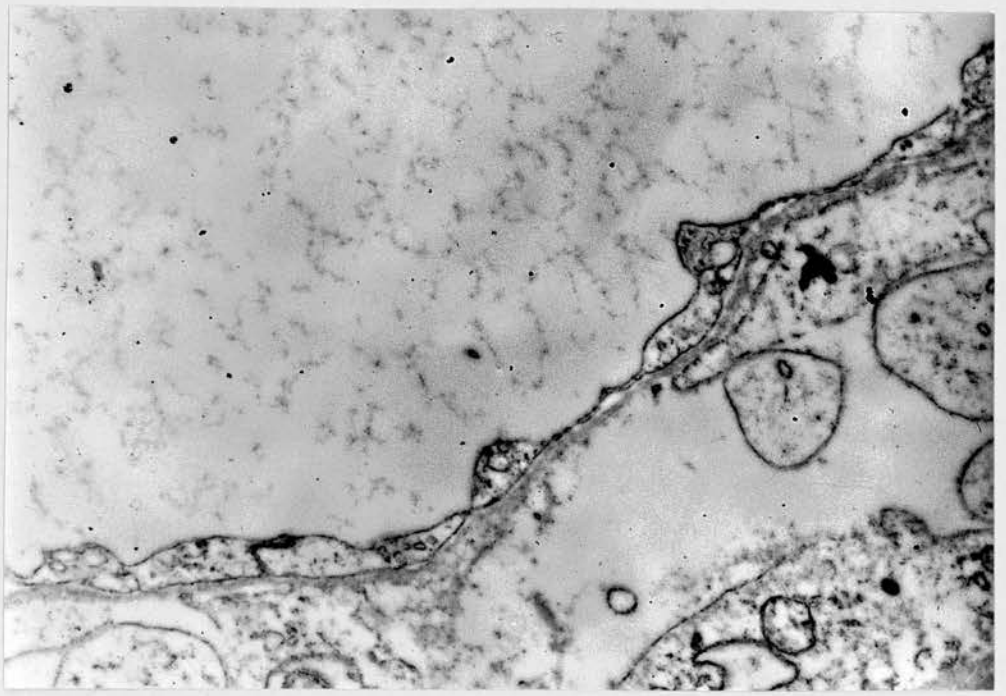


Fig. 148. An efferent vasae rectae capillary from the outer medulla of an albino rat. The endothelium is attenuated and interrupted by a number of very narrow fenestrae in two areas. An attachment belt is clearly seen on the left side. x 15,000

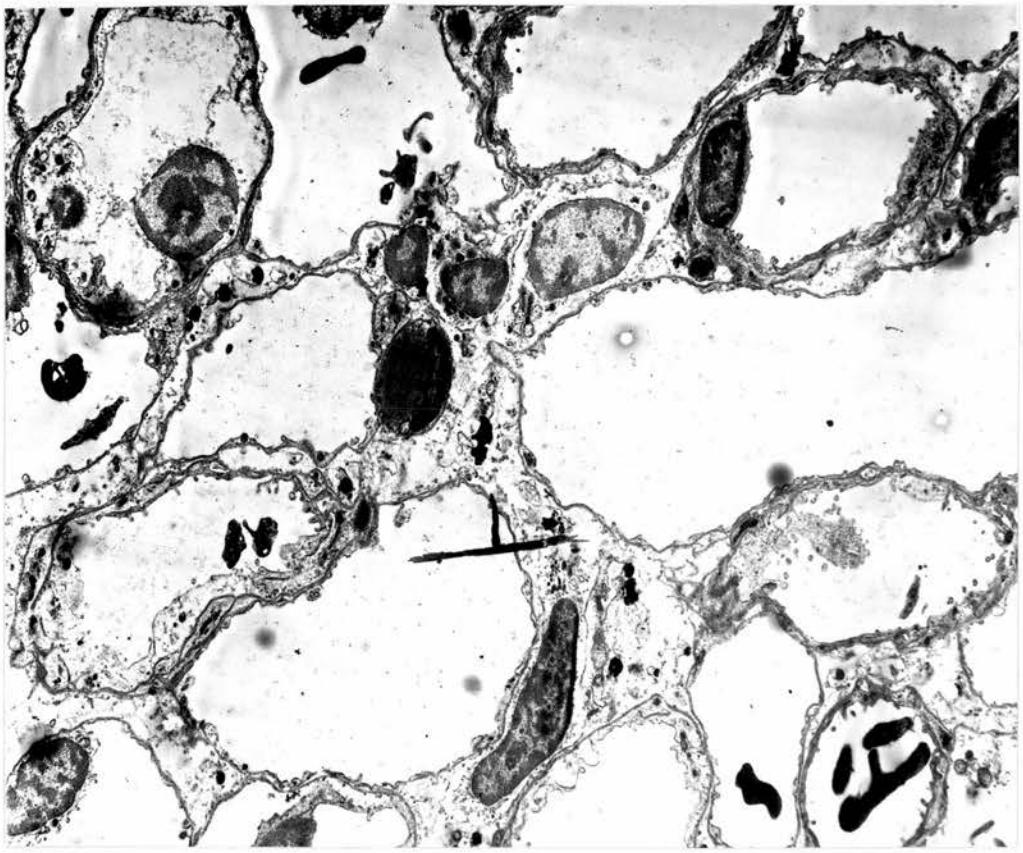


Fig. 147. The renal rete mirabile of capillaries from the inner strip of the outer medullary zone of an albino rat. This is a bunch of capillaries that run together away from the tubules. Note the two types of capillary: the descending "afferent" vasae rectae with a thick endothelial lining regularly alternating with the ascending "efferent" vasae rectae which have an attenuated, fenestrated endothelial lining.
x 1,350.

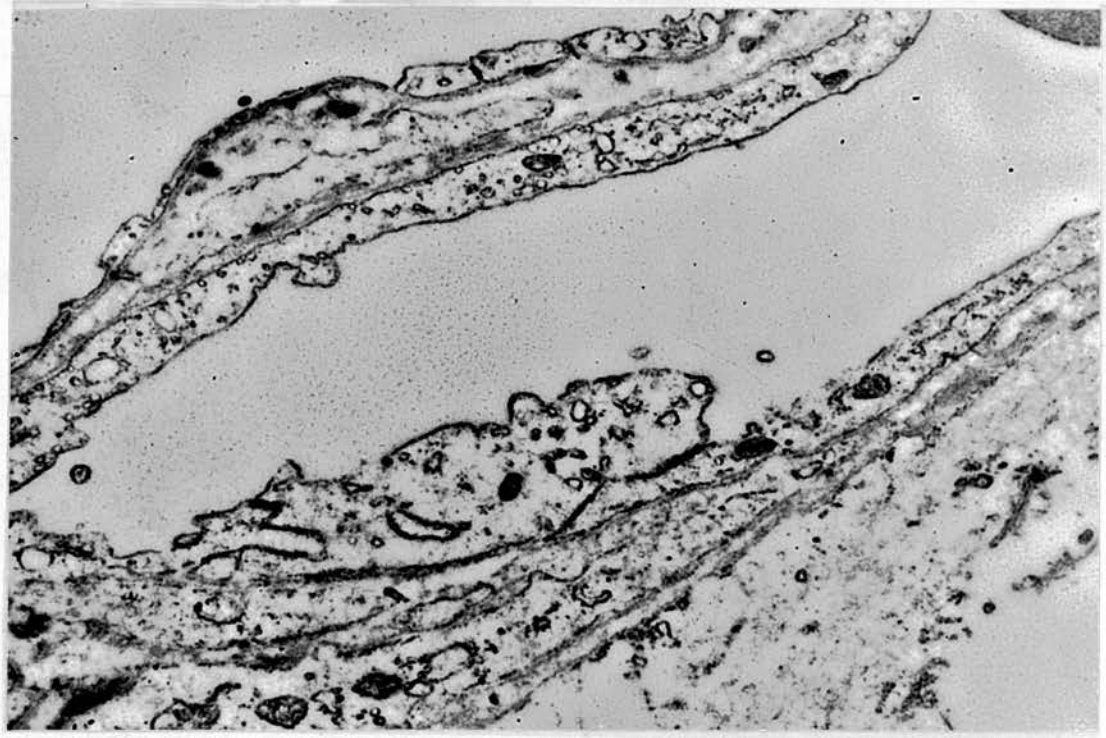


Fig. 149. Thick type of capillary from the outer medulla of an albino rat's kidney. x 16,000

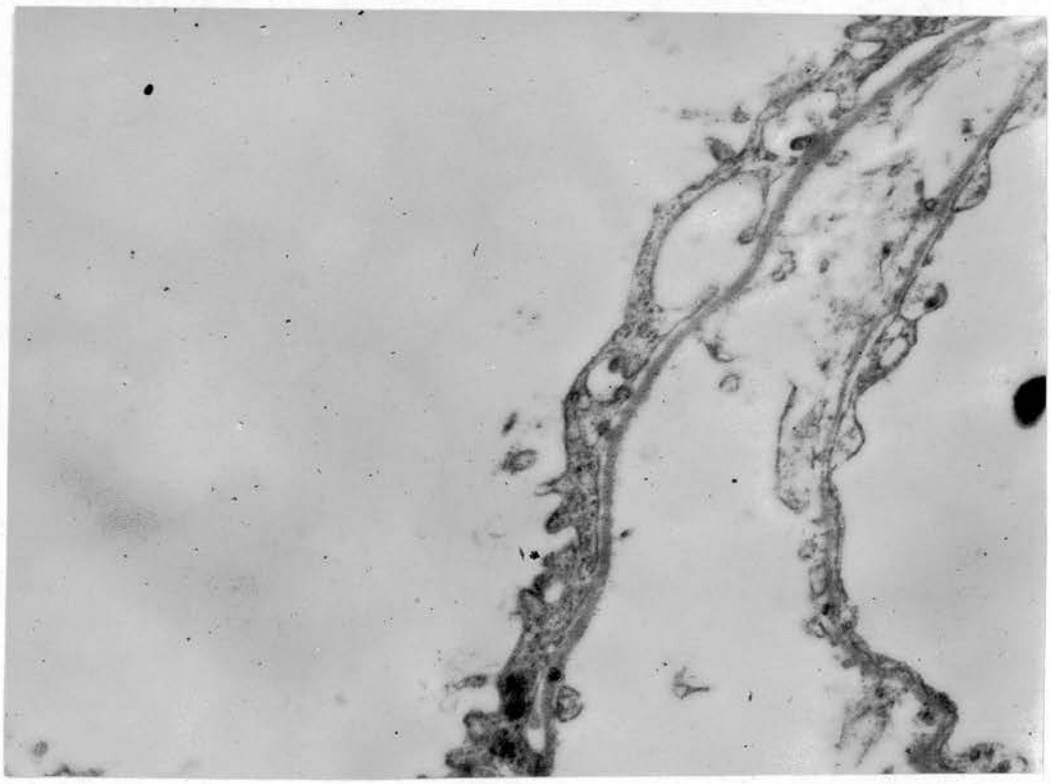


Fig. 150. Two capillaries from the outer medulla of an albino rat's kidney: the one on the right is lined by an attenuated, fenestrated endothelium, while the one on the left is of the thick type. x 16,000

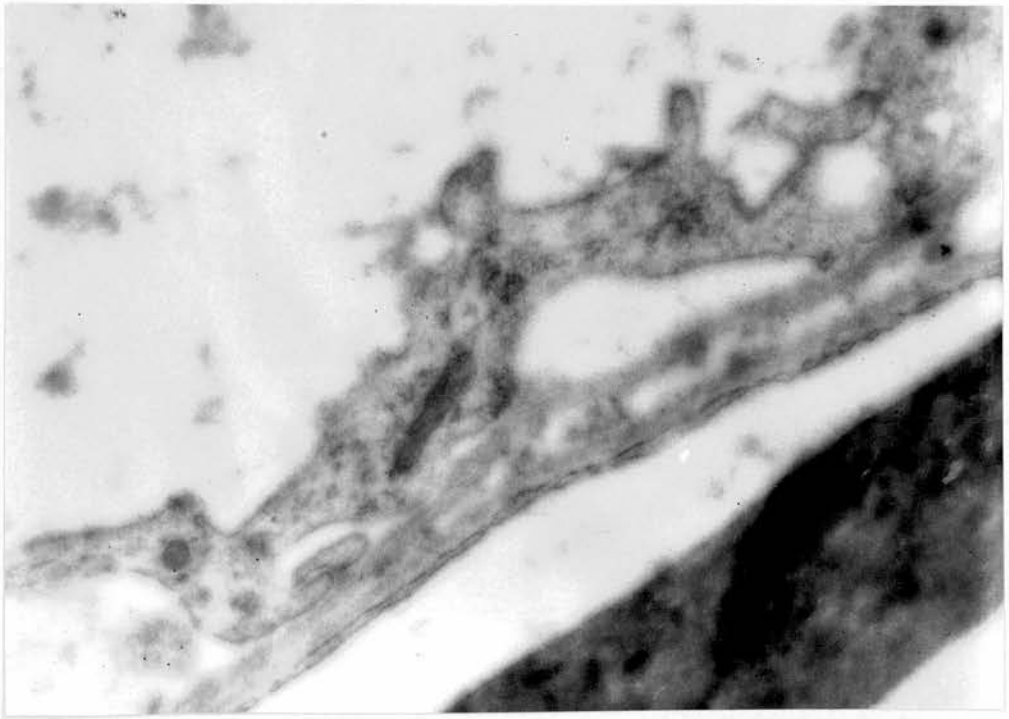


Fig. 151. The two types of capillary from the renal rete of an albino rat in contact. x 45,000

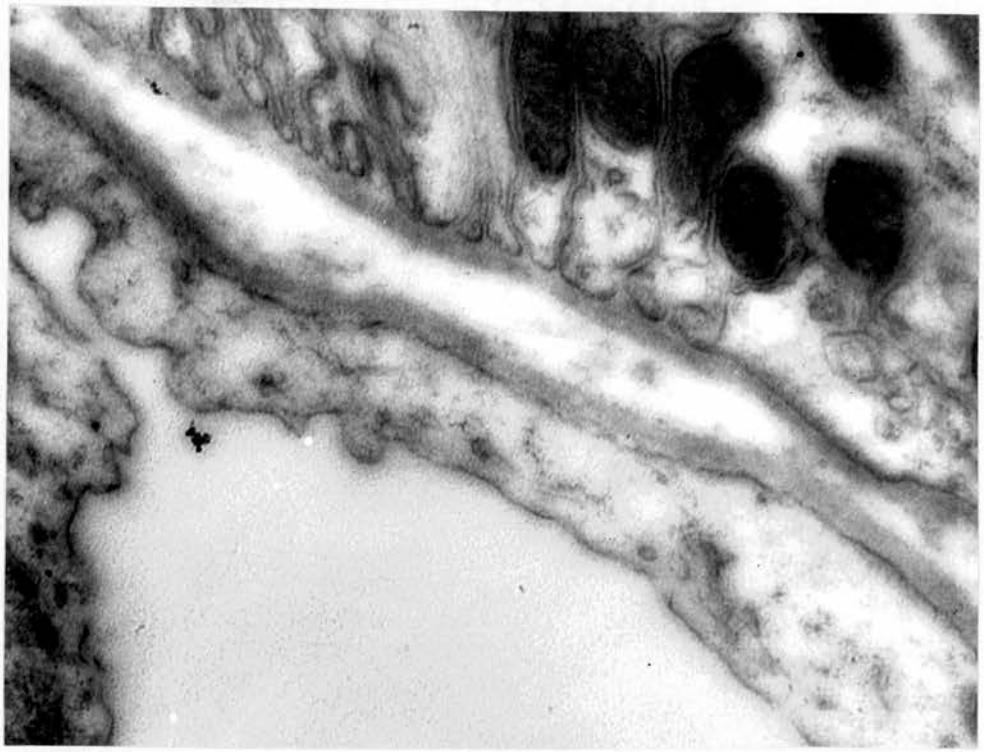


Fig. 152. The basal part of a thick segment of the ascending limb of a loop of Henle from the outer medulla of an albino rat. A definite space separates the tubular basement membrane from that of an adjacent thick type of capillary. x 45,000

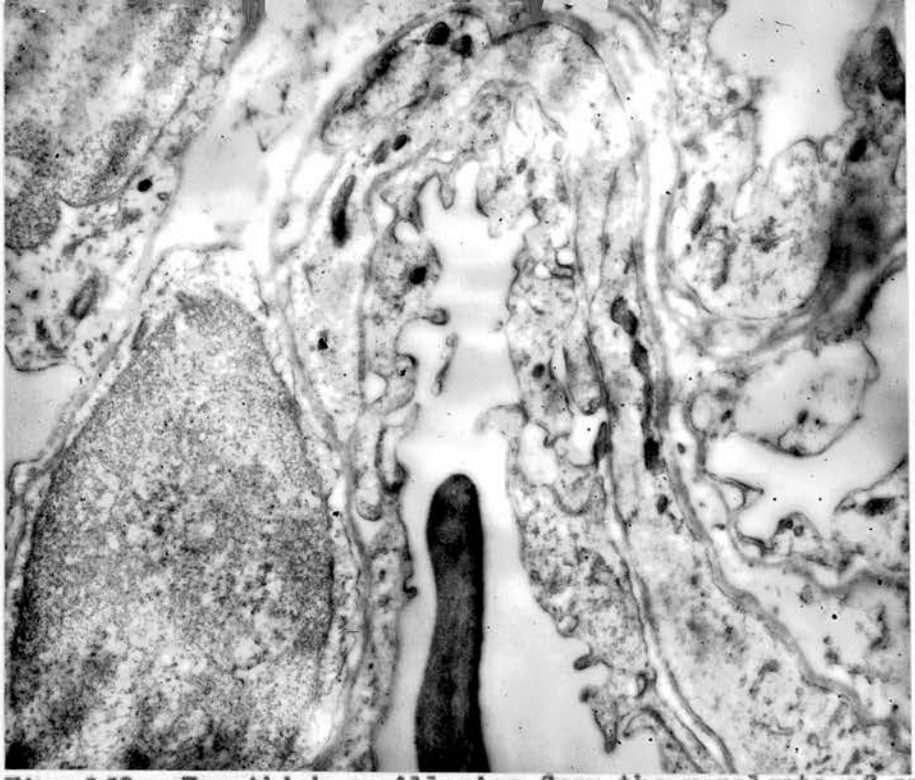


Fig. 153. Two thick capillaries from the renal rete of an albino rat. Pericytes can be clearly seen around the capillary on the left side. x 15,000

Fig. 154. The architecture pattern in the inner zone of the medulla is seen in this low power electron micrograph from an albino rat. Note the very wide space of ground substance in which the tubules and capillaries are embedded. Interstitial cells and fibres can be seen in the ground substance.
x 4,000

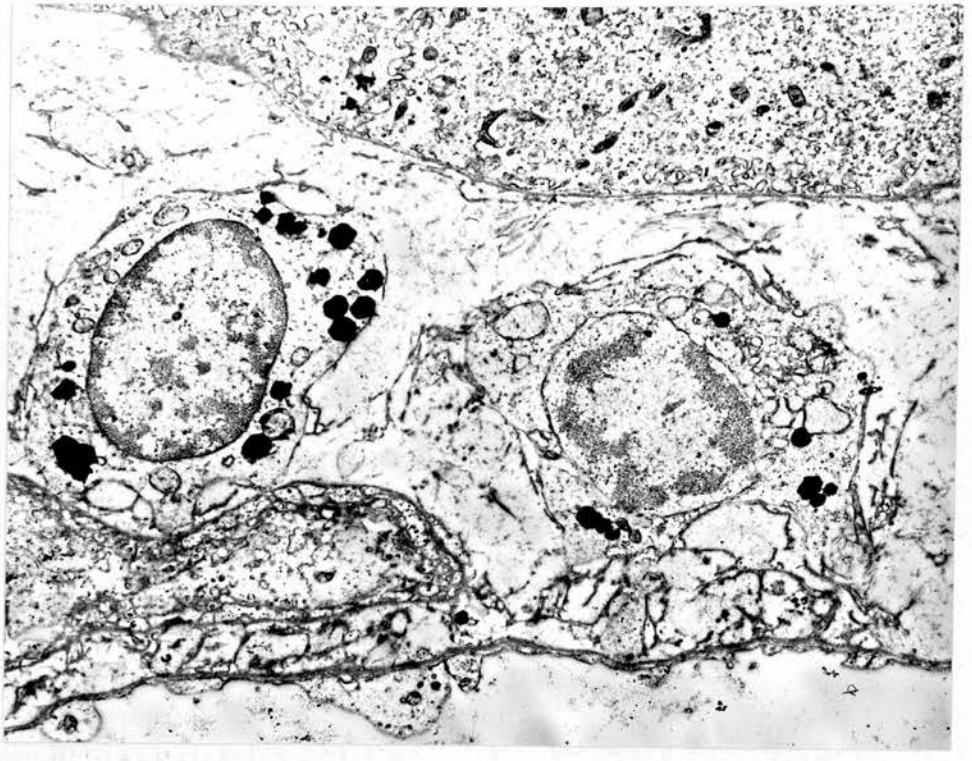


Fig. 155. Two interstitial cells from the inner medulla of an albino rat.

X 4.000

Fig. 156. An interstitial cell in the inner medulla of an albino rat showing three processes in contact with a capillary, a thin segment and a collecting tubule. x 9,000

with the socalled lysozyme. Sometimes, these granuoles can be seen free in the ground substance. Throughout the ground substance, connective tissue fibrillary structures are clearly seen. They form a loose meshwork which becomes wider and denser at the papillary apex (Fig. 155).

DISCUSSION.

According to the prevalent concepts of renal function and urine formation, about 1500 litres of blood (third of the cardiac output), circulates through the human kidneys each day. About 180 litres are filtered through the glomeruli daily, but only 1½ litres are excreted as the final urine. Therefore the vast majority of the glomerular filtrate is absorbed from the renal tubules, taking the route: tubule lumen, tubule epithelium, peritubular capillary. It is therefore quite evident that the great majority of the fluid in the nephron is not transported along its course but rather obliquely across it. About 80% of the glomerular filtrate takes this oblique direction in the proximal tubules, and 19% still maintains this oblique direction across the tubular epithelium in the distal and collecting tubules, and only a minute fraction less than 1% of the daily glomerular filtrate passes along the whole length of theurinary tubular system to the renal papilla, modified as it goes in a multitude of ways.

The tubules, in contrast to the glomerulus, consist of a closed layer of epithelial cells, therefore,fluid transport in them must be transcellular. The minute structure of the tubular epithelium entirely agrees with this function and is so constructed as to serve it best.

The presence of a large number of elongated microvilli in the proximal tubule results in an enormous increase in the surface area of the luminal side of the cell. This creates favourable conditions for reabsorption of fluid, particularly as in this segment of the nephron, there is no real free lumen to the tubule and the glomerular filtrate does not actually flow but "seeps" through (Fig. 123).

The polar lay-out of the cells of the proximal and distal tubules is emphasised by the spatial arrangement of the mitochondria. In the apical cytoplasm, just below the luminal surface, they are infrequent; while in the basal part, within the cytoplasmic processes, they occur in large numbers. Since the fluid, which enters at the lumen side, is returned to the surrounding blood space at the basal side, it may be assumed that the basal massing of the mitochondria is related to the latter process.

The plication of the basal cell membrane provides an enormous increase in the basal surface area of the cells. Rhodin (10) has placed great emphasis on the fact that a large number of the cytoplasmic layers between the plicated cell membrane, in one plane of section do not show any connection with the rest of the cell body. He interprets these as processes of neighbouring cells, and even goes so far as to explain the whole basal cell arrangement as composed of such digitations from adjacent cells. It must be admitted in Rhodin's favour, that interdigitations in the basal half of the cells are visible in large numbers in the epithelium of the proximal and distal tubules and that these interdigitations arise from the invagination of the above mentioned cytoplasmic layers one with another. On the other hand, it must be emphasised that a single lamella that seems separated in one plane of

section, may prove in another to originate from the same cell, as was shown by Ruska and Coworkers (11) by means of serial sections. But even without this, a single plane of section often reveals several adjacent cytoplasmic lamellae which certainly belong to one and the same cell. I, therefore, agree with Theones (14) that the increase in the basal cellular surface area is the guiding principle for this peculiar plication of the basal cell membrane. The idea suggested by Rhodin of interdigitation does not conflict with this at all. It may indeed be regarded as a necessary consequence of the plication principle, based on spatial and cytoarchitectural considerations. As far as function is concerned, it is essential to place the emphasis not so much on interdigitation as on the increase in cellular surface area and the consequent formation of the basal labyrinth, that extracellular space, closed towards the cell, open towards the basement membrane, which is only bounded on the side of the capillary lumen by the basement membrane.

It seems reasonable to suppose that the intimate contact between mitochondria and cell membrane provides particularly favourable conditions for fluid transport through the cell membrane. The process of reabsorption, consists of the <u>active</u> transport of substances through the cell membrane without, or against an osmotic gradient into the basal labyrinth to provoke an osmotic pressure there. This, in turn, produces a <u>passive</u> afterflow of water, predominantly from the tubular epithelium. Though an influx of water from the capillaries is theoretically possible, it does not occur because the amount of flow is proportional to the surface area and the surface area of this basal labyrinth on the capillary side is negligible in comparison with that on the tubular side. This active

transport of fluid at the basal end of the cell presumably also maintains the fluid transport within the cell and in turn the inflow of fluid from the lumen of the tubule via the microvilli. The basally situated sheet of mitochondria, according to this hypothesis, provides most of the energy for fluid transport from the cell and is also responsible for the uptake of fluid into the cell. Undoubtedly, some selection of the substances to be absorbed does take place, but this is largely a question of specific enzyme action and the principle of fluid transport is the same whatever substance is selectively absorbed.

This process of transepithelial fluid transport is indispensable for the whole process of tubular reabsorption. The "motor" for this transcellular transport is situated in the system of folds and fronds of the tubular epithelium, whichis well supplied with mitochondria and behaves as a physico-chemical apparatus for tubular reabsorption. In the basal labyrinth, osmotic pressure is converted into hydrostatic pressure, and the fluid diffuses through the basement membrane to enter the capillary lumen as soon as the hydrostatic pressure in the basal labyrinth exceeds that in the capillary.

This mechanism is admittedly tied up with the assumption that the basal labyrinth is expansile to a limited degree. Since every tubule is surrounded by its own basement membrane, which is a rigid, relatively non expansile structure, and the whole kidney is surrounded by a fibrous capsule, one might take this assumption for granted. This effect will be exaggerated when there is a diuresis, as all the adjacent tubules would tend equally to expand and would be competing for a limited space. Assuming that the capacity of the basal labyrinth for expansion is limited, then

the active transcellular fluid transport in conjunction with the mechanism of the osmotic-hydrostatic pressure transformation, would explain the whole problem of reabsorption. Even more, it would provide a surprising parallel to the filtration process in the glomerulus in so far as a hydrostatic pressure gradient would be responsible for fluid transport through basement membrane and endothelial fenestrae in both cases. The difference is that in the case of the glomerulus the energy producing the hydrostatic pressure comes from the heart, while in the proximal and distal tubules it comes from the enzymatic activity of the tubule cells.

When trying to relate the micromorphology of the nephron to its function in the medulla, the general arrangement of the tubules and capillaries becomes immediately highly significant. According to the recent views on renal physiology, the loops of Henle function as countercurrent multipliers, creating a hyperosmotic milieu in the interstitium of the medulla which allows water to diffuse passively from the collecting tubules as they traverse it. In the outer medulla, there is little interstitial space, and therefore, there is no anatomical basis for the formation of an interstitial sodium pool. In the inner medulla, on the other hand, ideal conditions for the formation of a hyperosmotic milieu of the so-called sodium pool exist. Here, on account of the presence of a wide interstitial space, an exchange of solute or water between tubules or tubules and capillaries can only take place through the ground substance of the interstitium into which all tubules and blood vessels are individually embedded.

In the inner medullary zone only thin segments of the loop of Henle and collecting tubules with "light" cells are found. The epithelial

cells of these two types of tubules show many similarities; both are examples of relatively simple cells. Both have a little RNP granules, a few small mitochondria and very few and shallow basal cell foldings. All these features imply a low rate of oxidative metabolism and of vital activity, and sharply contrast with the cytological features of the epithelium of either theproximal or the distal tubule. However, the collecting tubule cells are three to six times as high as the cells of the thin segment and show slightly more regular plications of the basal cell membrane. This might indicate that they pursue some slightly more active functions than the cells of the thin segment of Henle's loop.

The question of the site and significance of the dark cells of the collecting tubules has been controversial. Rhodin (10) considered them to be identical with the epithelium of the arched collecting tubules, and believed that they represent cells that have been displaced, as it were, into the medulla. He found them mainly in the cortical collecting tubules and stated that they were absent in the papillary region. Lapp (4), on the other hand, stated that they are present in a very limited stretch along the course of the collecting tubules. He found them only in the outer half of the inner zone of the medulla and continuously failed to find them in the outer medulla or in the inner half of the inner medullary zone. He disagreed with Rhodin's view that they are morphologically, and possibly functionally, similar to the cells connecting the distal convoluted tubule to the collecting duct. Clark (1) believed that the dark and the light cells of the collecting tubule are the same type of cell in different functional states. However, I have found them rather frequently in the cortical collecting tubules and they gradually

diminished in number as the medulla is approached, and were practically absent in the inner medullary zone. I believe that there is a very gradual transition in cell morphology as one proceeds from the distal convoluted tubule to the tip of the collecting duct at the apex of the papilla, with a considerable degree of overlapping. This might indicate that the very active function of the distal tubules tails off for quite a distance along the collecting tubule before it becomes completely replaced by the purely passive conducting function of the Ducts of Bellini.

The morphological arrangement of the tubules in the outer zone of the medulla has not been taken into consideration by the physiologists trying to overcome many of the difficulties concerned with the details of the hair-pin countercurrent theory. In the loop of Henle, the transition from thin to thick segment, occurs much nearer the cortex in the descending than in the ascending limb (Diagram 3). For the juxtamedullary nephrons, with long loops - which according to this theory are the ones responsible for the creation of a hyperosmotic milieu in the papilla - three different zones can be recognised, consecutively from outside inwards (Diagram 3).

I. In the outer strip of the outer medullary zone, the pars recta of the proximal tubule (thick and active) is juxtaposed to the pars recta of the distal tubule (thick and active).

II. In the inner strip of the outer medullary zone, the thin descending limb is juxtaposed to the thick ascending limb.

III. In the inner medullary zone, both descending and ascending limbs are thin.

This morphological arrangement which has been very briefly illustrated or mentioned in many standard text books of histology (e.g. 6) for the last

few decades since the investigations of Peter in 1909 (9), has been entirely confirmed in this electron microscopic study.

According to Wirz's theory, the ascending limb of the loop actively transports Na^+ into the interstitium, while the descending limb behaves passively in allowing water to diffuse out into the interstitium according to the osmotic gradient created by the presence of Na^+. When one tries to apply this to the morphology as just described, it becomes immediately obvious that two important difficulties arise. Firstly, the lower half of the ascending limb consists of simple flat cells that cannot be perceived to have any active function beyond that of their maintenance metabolism, and the thick segment of the ascending limb must be regarded solely as the active part. Secondly, the pars recta of the proximal tubule is considered by the theory to be a passive segment, as the rest of the descending limb, while from the cytological appearance of its cells, it must be very active. In addition, in between the two columns of urine flowing in opposite directions, there are extraordinarily complicated biological structures, mainly the peculiar interstitial cells found in abundance in the inner zone of the medulla, and these have not been accounted for in the theory.

Finally, the arrangement of the capillaries in the renal medulla described here is novel and very significant. Rhodin in his classical studies on the renal tubules briefly mentioned the fact that sometimes the tubules share their basement membrane with adjacent capillaries. Pease (8) described the endothelium of the "peritubular capillaries" as much attenuated and perforated by holes which resemble those of the glomerular capillary but differ in that they are smaller in diameter and more widely spaced. Siadat-Pour (13) gave detailed information about the basement membranes and their

relation to epithelium and endothelium, and stated that the medullary capillaries possess a perforated endothelium. Longley and co-workers (5) distinguished two types of capillaries on the basis of theminute structure of the endothelium in the region of the vasae rectae. In this study, the existence of two types of capillaries has been independently arrived at. In addition it was clearly shown that the two types exist only in the outer medulla, while all the capillaries in the papilla have an attenuated fenestrated endothelium. In the outer medulla, it was shown that most capillaries run in a bundle or tuft away from the tubules, while in the inner medulla they are evenly dispersed among the tubules in the ground substance. They reach a lower level than the loops of Henle, so that at the extreme apex of the papilla, only collecting tubules and thin capillaries are to be found. The unusual abundance of capillaries in the inner medulla has been stressed, an abundance which is quite out of proportion to the relatively low oxygen requirements of this part of the kidney. The constant relation of the thin fenestrated type of capillaries to the tubules of the loop of Henle, thick or thin, and the rarity of such relationship to the collecting tubules has been mentioned. The endothelium of the thick type of capillary is very similar to that of the arterioles and meta-arterioles and I believe that these capillaries carry the descending stream of blood, while the fenestrated ones represent the ascending venular capillaries.

The fact that in the outer medulla, the thin and thick capillaries regularly alternate in the capillarytuft (Fig. 147), giving an appearance very similar to the rete mirabile of the swim bladder of fish together with the fact that the different types of capillary frequently share a common basement membrane (Fig. 151) produce the optimum morphological requirements for them to function as counter-current exchangers. This resemblance in the

architectural arrangement has also been shown to extend to their submicroscopic structure. Various investigators (2, 3) have reported differences in the ultrastructure between the afferent and efferent capillaries in the rete mirabile of the swim bladder of fish. They have found the afferent capillaries to be lined with an endothelium varying in thickness from 2 to 4 μ; while the efferent capillaries are lined by an attenuated fenestrated endothelium.

Nowhere in nature is countercurrent exchange more strikingly developed nor more clearly illustrated than in the swim bladder of deep-sea fish. The function of the rete mirabile in this situation is to prevent the loss of oxygen from the fish's air bladder. A deep-sea fish keeps its swim bladder filled with gas which is more than 90% oxygen. At depths of 9.000 feet or so, it must maintain an oxygen pressure amounting to 200 to 300 atmospheres (12). On the other hand, the oxygen pressure in the bladder's surroundings - in the fish's blood stream and in the sea water outside - no more than a fifth of an atmosphere. However, very little oxygen escapes from the swim bladder wall to the rest of the fish's body, because the outgoing venous capillaries, highly charged with oxygen, give it up to the adjacent incoming arterial capillaries. There is a net-work of thousands of looping capillaries so closely intermingled that diffusion of oxygen from veins to arteries goes on at an extremely high rate.

The structural and architectural resemblance between the vasae rectae in the mammalian kidney and the capillaries in the swim bladder of fish suggests that they are very similar in function. However, in addition to the capacity of the renal rete for an efficient countercurrent diffusion, it is reasonable to suggest that they have a more complex function than the

mere passive exchange of diffusible material between the two sets of vessels. The structure of the endothelium of the arterial capillaries goes beyond that required for a simple passive function. Moreover, a striking difference in histochemical activity between the efferent and afferent vessels in the medullary vascular bundles of the rat has been observed; the descending arterial capillaries show intense estrase activity in their endothelium, while the ascending venular capillaries show none (5). The endothelium, particularly of the arterial capillaries, can be conceived to modify their permeability so as to regulate the molecular and ionic species subject to countercurrent exchange. If, in addition, the endothelium is able to transport actively any substance from or into the blood flowing through the arterial capillaries, then for that substance, the countercurrent exchanger would become a countercurrent multiplier, able to contribute, not only to the maintenance of, but to the creation of the hyperosmolarity of the renal papilla.

REFERENCES.

1. Clark, S.L.
Cellular differentiation in the kidneys of newborn mice studied with the electron microscope.
J. Biophys. & Biochem. Cytol., 3: 349, 1957.

2. Fange, R.
Acta Physiol. Scand., 30:(Supp), 110, 1953.

3. Fawcett, D.W. and Wittenberg, J.
The fine structure of capillaries in the rete mirabile of the swim bladder of Opsanus tan.
Anat. Rec., 133: 274, 1959.

4. Lapp, L.
Elektronenmikroskopische Untersuchengen Zur Funktion des Nierenmarkes der Ratte.
Verh. Deutsch. Gesellsch Fur Path., 44: 294, 1960.

5. Longley, J.B., Banfield, W.G. and Brindley, D.C.
Structure of the rete mirabile in the kidney of the rat as seen with the electron microscope.
J. Biophys. & Biochem. Cytol., 7: 103, 1960.

6. Maximow, A.A. and Bloom, W.
A Textbook of Histology.
7th Ed., PHILADELPHIA. W.B. Saunders Comp., 1957.

7. O'Dell, R. and Schmidt-Nielsen, B.
Concentrating ability and Kidney structure.
Fed. Proc., 19: 366, 1960.

8. Pease, D.C.
Fine structures of the kidney seen by electron microscopy.
J. Histochem. & Cytochem., 3: 295, 1955.

9. Peter, K.
Untersuchungen Uber Bau und Entwickelung der Niere.
Jena: Fischer, 1909.

10. Rhodin, J.
Anatomy of kidney tubules.
International Review of Cytology, Vol. 7, p. 485-534.
Ed: Bourne, G.H. and Danielli, J.F. Academic Press Inc., Publishers, NEW YORK, 1958.

11. Ruska, H., Moore, D.H. and Weinstock, J.
The base of the proximal convoluted tubule cells of rat kidney.
J. Biophys. Biochem. Cytol., 3: 249, 1957.

12. Scholander, P.F.
The wonderful net.
Scient. American, 196: (April), 1957.

13. Siadat-Pour, A.
Die Ultrastructur des Blut-Harnweges in verschiedenen Abschnitten des Nephrons bei der Maus.
Beitr. path. Anat., 120: 382, 1959.

14. Thoenes, W.
Die Mikromorphologie des Nephron in ihrer Beziehung zur Funktion. Teil I: Funktions einheit: Glomerulum-Proximales und distales Konvolut.
Klin. Wschr., 39: 504, 1961.

15. Ullrich, K.J., Kramer, K. and Baylan, J.W.
Present knowledge of the countercurrent system in the mammalian kidney.
Prog. Cardiovasc. Dis., 3: 395, 1961.

ELECTRON MICROSCOPIC STUDY OF THE MECHANISM OF DILUTION AND CONCENTRATION OF THE URINE AND THE ACTION OF THE ANTIDIURETIC HORMONE IN THE MAMMALIAN KIDNEY.

ELECTRON MICROSCOPIC STUDY OF THE MECHANISM OF DILUTION AND CONCENTRATION OF THE URINE AND THE ACTION OF THE ANTIDIURETIC HORMONE IN THE MAMMALIAN KIDNEY

Introduction.

Nearly a century has elapsed since Claude Bernard, among the most notable of nineteenth-century physiologists, first pointed out that the true medium in which we live is neither air nor water, but the plasma that bathes all the tissue elements. This "milieu interieur", as he later called it, is so isolated from the world that atmospheric disturbances cannot alter it or penetrate beyond it: "It is as though the organism had enclosed itself in a kind of hothouse where the perpetual changes in external conditions cannot reach it". It was Bernard's view that we achieve a free and independent life physically and mentally, because of the constancy of the composition of our internal environment.

In Bernard's time the chemistry of living organisms was poorly known and afforded only a meagre insight into the complexity of the internal environment, but as the modern sciences of biochemistry and physiology have added chapter after chapter to this subject, this new knowledge has only emphasised the importance of his generalisation.

The lungs serve to maintain the composition of the extracellular fluid with respect to oxygen and carbon dioxide, and with this their duty ends. The responsibility for maintaining the composition of this fluid in respect to other constituents devolves on the kidneys. It is no exaggeration to say that the composition of the body fluids is determined not by what the

mouth takes in but by what the kidneys keep: they are the master chemists of our internal environment, which, so to speak, manufacture in reverse by working it over completely some fifteen times a day. When, among other duties, they excrete the ashes of our body fires, or remove from the blood the infinite variety of foreign substances that are constantly being absorbed from our indiscriminate gastrointestinal tracts, these excretory operations are incidental to the major task of keeping our internal environment in an ideal, balanced state. Our bones, muscles, glands, even our brains, are called upon to do only one kind of physiological work, but our kidneys are called upon to perform an innumerable variety of operations. Bones can break, muscles can atrophy, glands can loaf, even the brain can go to sleep and not endanger our survival; but should the kidneys fail in their task neither bone, muscle, gland nor brain could carry on (48).

Recognising that we have the kind of internal environment we have because we have the kind of kidneys that we have, we must acknowledge that our kidneys constitute the major foundation of our physiological freedom. Only because they work the way they do has it become possible for us to have bones, muscles, glands and brains. Superficially, it might be said that the function of the kidneys is to make urine; but in a more considered view, one can say, as Homer Smith has rightly put it (48) that the kidneys make the stuff of philosophy itself.

All marine invertebrates are isosmotic with their environment, in other words, their body fluids have the same salt content and the same osmotic pressure, as the sea water in which they live. The maintenance of salt and water balance is therefore a relatively simple matter. Excess salt is excreted through the respiratory epithelium, and the problem of excreting water per se does not exist. In them, the kidney is a

glandular organ concerned chiefly with the excretion of metabolic waste products that cannot escape from the body by simple diffusion. It may be assumed that such was the situation in the marine ancestor of the vertebrates(48)The fact that the theater of evolution of the early vertebrates was in fresh rather than salt water had momentous consequences; the provertebrate had to become adapted to life in a fresh medium. Such adaption demanded primarily the development of a system for the effective elimination of water which, by virtue of the osmotic gradient, continuously entered the animal's internal medium, which was hypertonic to the medium in which it lived. Inadequacy of such a system would inevitably lead to leeching of the organism of its salts. The kidney came into a new and important role: it became the chief route for the excretion of water from the body, an operation that had to be carried out without loss of salt, and thus it came to be responsible for the regulation of the composition of the internal environment in respect to both water and salt, as well as for the excretion of waste products.

Water was available in excess to the provertebrate, but the concentration of sodium chloride in this water was low and highly variable and it is not too venturesome to think that the tenacious conservation of salt is one of the most primitive - if not the most primitive of functions in the vertebrate kidney. The evolution of an equally tenacious conservation of water posed a problem to which entirely different solutions were to be found by the amphibia, reptiles, birds and mammals - until after four hundred million years a small, nocturnal rodent can live in a desert burrow without water and the scientist can write in rhythmic heptads:

Salt and Water.

In the beginning the abundance of the sea
Led to profligacy
The ascent through the brackish waters of the estuary
To the salt-poor lakes and ponds
Made immense demands
Upon the glands
Salt must be saved, water is free.

In the never-ending struggle for security
Man's chiefest enemy,
According to the bard of 'Stratford on the Avon'
The banks were climbed and life established on dry land
Making the incredible demand
Upon another gland
That water, too, be saved.

Maurice B. Strauss, November 23, 1951.

The transition to terrestial existence in no way diminished the power to counteract hyperhydration inherited from fresh water ancestors. At the same time the animals that emerged on dry land acquired the power to combat dehydration by effecting, when necessary, considerable reduction in the excretion of osmotically free water. However, this conservation is imperfect in all vertebrates except birds and mammals. Frogs kept in air at 18°C lost 22% of their body weight in 6 hours. Toads, which are more terrestial than frogs, and counteract dehydration more effectively; lost 14% only, because of the greater impermeability of their integuments to water (13). In their urine these animals have reduced the excretion of osmotically free water sharply, but are unable to excrete urine which is completely free from this fraction.

Even animals which are completely terrestial such as reptiles, when subjected to dehydration, still excrete small quantities of free water taking it from tissues already suffering from a deficiency. Only birds and mammals

have been completely freed from such inevitable excretion. When there is restriction of water intake, the urine serves solely for the excretion of metabolic products, and contains no osmotically free water. Moreover among all vertebrates, birds and more particularly mammals, have the power to concentrate the urine against an osmotic gradient,so that during dehydration it becomes hypertonic in relation to the blood. Smith has suggested that this concentrating capacity of the mammalian kidney may have played an important role in our evolution, and specifically in the ultimate domination of the mammals over the dinasours and other reptiles at the end of the Mesozoic Era (48).

The happy facility which enables the mammalian organism, in addition, to vary, over rather wide ranges, the relative proportions of urinary water and solute is a phenomenon of manifest teleologic advantage and constitutes perhaps the major line of defence with regard to homeostasis of volume and tonicity of the body fluids. The osmotic pressure of the extracellular fluids, and presumably of the cells they bathe, is punctiliously guarded at values of approximately 280 to 300 mOsm/Kg. water. The cellular milieu of the human kidney, however, must somehow cope with the fluid contents within its tubules, which between the extremes of maximum diuresis and antidiuresis ranges from under 50 to more than 1,300 mOsm./Kg. water, that is, roughly one sixth to over four times the osmotic pressure of the plasma. The kidney of other mammals may perform even more spectacularly: the urine within the collecting ducts of certain desert rodents that subsist largely upon dry diets may attain concentrations 17 times that of the plasma (42).

The flexibility of the urinary function of the kidney and the mechanisms

underlying it, have understandably been subjected to considerable experimental scrutiny and various conclusions have been deduced and formulated into hypotheses and theories to explain the mechanisms of dilution and concentration of the urine. In reviewing the historical development of the various theories, it is interesting to note how often the concentrating function of the kidney has been linked to the loop of Henle. Such a relationship was suggested in 1909 by Peter (36) who noted a correlation between the maximal concentration of the urine achieved by various mammals and the length of the thin segment of the loop of Henle in the kidneys of such mammals. In 1927 Crane (7) pointed out that only mammals and birds can form concentrated urine and that it is only in these phyla that thin segments of the loops of Henle occur. Closely related to this observation were the experiments of Burgess et al (6), which showed that it was only in birds and mammals that antidiuretic hormone increased the tubular reabsorption of water. In fact, on the basis of these experiments Burgess et al were led to the almost prophetic hypothesis that the urine was concentrated in the loop of Henle and that antidiuretic hormone had its locus of action upon this segment of the nephron.

This hypothesis, however, seemed to be invalidated by the classic studies of Walker et al in 1941 (57), which demonstrated that the tubular urine obtained by direct micropuncture from the distal convolution of the nephron of the rat was at most iso-osmotic and centainly was not hyper-osmotic as would be expected if the final concentrating operation occurred in the loop of Henle. An alternate mechanism for concentrating the urine was then developed by Homer Smith and his co-workers Wesson and Anslow (45,46,59, 60) and, until quite recently was the most popular and the most

plausible theory of the mechanisms of urinary concentration and dilution.

The composition of the urine finally reaching the bladder depends upon the specifically renal operations of filtration at the glomerulus, and reabsorption from, and secretion into the tubules of water and solutes. The structural variations along the course of the individual nephron are such as to imply specific functional properties for each discrete segment. Current concepts of the functional geography of the nephron originate largely from the works of Richards (37), Walker (57) and their colleagues. Utilising micropuncture and microanalytic techniques, these workers established that the fluid in Bowman's capsule of the amphibian kidney was essentially free of protein and that its composition was characteristic of an ultrafiltrate of plasma with respect to osmotic pressure, electrical conductivity, pH, glucose, creatinine, inulin and electrolytes. Extending these techniques from the amphibian to the mammalian kidney, Walker et al (57) were able to puncture and analyse the fluid from various portions of the proximal tubule of the rat, guinea pig and opossum. They determined that by the end of the proximal tubule (a) virtually all of the glucose and phosphate of the glomerular filtrate had been reabsorbed (b) the volume of the glomerular filtrate had been reduced by approximately 80% (c) the creatinine concentration had increased almost fivefold, but (d) the total osmotic activity remained equal to that of plasma. Since creatinine is apparently neither secreted nor reabsorbed from the tubule to any significant extent, intraluminal concentration of this substance can be achieved only by the removal of water. Net osmotic activity, however, did not change; reabsorption of water, therefore, must have been accompanied by an amount of solute in such proportion as to maintain isotonicity between the reabsorbate,

the fluid residual in the tubule, and the plasma. Since the activity of sodium and its associated anions contributes the bulk of the osmotic pressure of plasma and hence of the glomerular filtrate, it follows that proximal reabsorption of water proceeds together with sodium salts in roughly their concentration in the extracellular fluids.

Further information about the nature of these proximal processes was contributed by Wesson, Anslow and Smith (58, 60) in 1948. These workers induced osmotic diuresis in dogs by administering hypertonic solutions of mannitol, a non-reabsorbable solute, intravenously. The resultant diuresis approximated, per minute, two thirds of the glomerular filtration rate. Such a very rapid flow seemed to preclude any marked modification by more distal structures, so that the final bladder urine was assumed to represent the composition of the fluid at the end of the proximal segment (45). The excreted urine contained up to 65% of the filtered water but no more than 27% of the filtered sodium. This implies that reabsorption of sodium ion is independent of the reabsorption of water and that it proceeds against a concentration gradient and is most likely an active process.

Based largely on the foregoing, the concept of Smith, Wesson and Anslow concerning events in the proximal segment is (1) a primary active removal of sodium followed by (2) an obligatory passive diffusion of water along the osmotic gradient resulting from the active movement of solute, so that the osmotic urine to plasma ratio (U/p) remains close to unity. (The U/p ratio expresses the relationship between the concentration of a substance in the urine and its concentration in plasma, affording an index of the extent to which a substance is diluted ($U/p < 1$) or concentrated ($U/p > 1$)). Thus,

the net accomplishment of operations within the proximal tubule is to return to the circulation the bulk of the filtered water and solutes which metabolic necessity dictates should be eliminated. This process of "routine conservation", reduces the volume of the glomerular filtrate by about 80% - 85%.

The loop of Henle was thought by Smith and his collaborators to represent a segment where osmotic equilibration could be completed, and its thin epithelium seemed ideally suited to this function. The view that it is the site of urinary concentration was discarded, largely because of the argument that its epithelium is too thin to perform osmotic work (45). The finding by Walker et al (57) in three punctures of the distal tubule of concentrating rat kidneys, that the fluid therein may be somewhat hypotonic to plasma, indicating that the filtrate which has already traversed the loop of Henle is not concentrated, has been also brought forth as further supporting argument against the idea that concentration of the urine occurs within the loop. According to Smith's theory, the active process in the proximal tubule of primary movement of sodium, might slightly outstrip the passive reabsorption of water, rendering the fluid hypotonic; the thin limb, composed of epithelium permeable to water, would allow diffusion of water so that isotonicity could be re-established.

The processes of glomerular filtration, proximal reabsorption and equilibration in the loop of Henle, resulting in an isosmotic fluid, would seem to be fixed and, in an osmotic sense, unselective. To the distal tubule and collecting duct, then, in this scheme, are detailed the discriminatory mechanisms which determine the ultimate concentration or dilution of the urine. In man, the urine flow may range from less than 0.5 ml. per minute

under conditions of dehydration to over 20 ml. per minute during maximal water diuresis. This latter figure represents in normal man about one-sixth to one-eighth of the glomerular filtration rate, a value not exceeded even in severe diabetes insipidus (44) and affords clinical confirmation of the calculation by Walker et al (57), that about 85% of the glomerular filtrate is reabsorbed before it enters the distal system. Reduction of urine flow to minimal rates implies potential reabsorption within the distal system of about 19 ml. per minute, an operation Smith calls "Facultative reabsorption", mediated largely under the influence of antidiuretic hormone (ADH) of the posterior pituitary gland, and implying, contrary to the "obligatory reabsorption" in the proximal system, that the movements of solute and water are not always isosmotic.

The isotonic fluid in the distal system can be rendered hypotonic by one of two conceivable mechanisms: one is the addition of water without solute to the tubular fluid, and the other is the removal of solute without water from the tubular fluid. There is no conslusive evidence that tubular secretion of water does occur, and several reasons have been presented (59) for regarding this as an unnecessary and at present untestable hypothesis. That the urine, during water diuresis in circumstances where retention of sodium is maximally stimulated, may be virtually sodium-free argues strongly for a distal process for the removal of osmotically active solute and implies an active transport of such solute against even greater chemical and osmotic gradients than obtain during proximal reabsorption. The epithelium of the distal tubule in this theory, in the absence of any activity of ADH i.e. in sustained water diuresis or in diabetes insipidus, is virtually waterproof, and so water is restrained from following in the wake of the actively

transported sodium. For the first time, then, water is retained in the tubular lumen without being completely obligated by an equivalent amount of osmotically active solute. This is not to imply that there is no osmotic activity in the tubule. Urea and other organic metabolic end-products are reabsorbed only to a small and variable extent as the glomerular filtrate traverses the nephron; these waste products must be excreted. One can therefore conceive of a dilute urine as composed of two moieties: a small volume of isosmotic fluid, containing the solutes that were not reabsorbed plus a large amount of osmotically unobligated water, so-called "free-water", resulting from the abstraction of sodium and attendant anions. It is this "free-water" that renders the glomerular filtrate hypotonic to the plasma and accounts for the dilution of the urine.

In order to afford a more quantitative basis for the exploration of the renal events concerned with concentration and dilution of the urine, Smith and Wesson and Anslow (47, 59) divide the urinary water into several fractions as shows by the following formulae:

$$C_{osm} = \frac{Uosm}{Posm} \cdot V \qquad (1)$$

$$C_{H2O} = V - Cosm \qquad (2)$$

$$T^{c}_{H2O} = Cosm - V \qquad (3)$$

V represents urine flow in millilitres per minute, and Uosm and Posm represent the osmolal concentration of urine and plasma, respectively, as determined crysoscopically, generally by means of freezing-point depression. The first fraction is the osmolal clearance, Cosm, and it represents the volume of urinary water required to contain the urinary solutes in a concentration isotonic with the concomitant plasma, so that U/P = 1. The second fraction is the net excess or deficit of water in the urine over

and above the osmolal clearance. This is referred to as the free-water clearance which will be positive under conditions of water diuresis in the absence of ADH and negative in antidiuresis under the influence of ADH. Rather than speak constantly of a negative free-water clearance, the deficit of water resulting during the excretion of hypertonic urine is referred to as T^{c}_{H2O}, derived as shown in formula (3). It should be emphasised that these divisions are purely mathematical and are not meant to imply that the urine is in fact discretely fractioned in the functional operations of the nephron.

In summary, the process of urinary dilution may be visualised as follows: The glomerular filtrate, considerably reduced in bulk following operations in the proximal system, but still isosmotic, is presented to a site in the distal tubule which is virtually impermeable to water. Sodium salts are actively removed in this locus, leaving osmotically free water in the lumen, which renders the filtrate hypotonic to the plasma and results in a dilute urine. Implicit in this postulate is the dependence of urinary dilution upon the delivery to the diluting site of sodium and its salts: If none were delivered, none could be reabsorbed, and no free water could be left behind to effect dilution of the urine.

Under conditions of dehydration, or under the influence of ADH, the free water liberated by the distal reabsorption of solutes must be reabsorbed and an additional amount of water must also be removed in order to render the urine hypertonic. Thus, from an isotonic filtrate, to produce a dilute urine, there is active reabsorption of solute without water; to elaborate a hypertonic urine, there must be an abstraction of water without solute. When a person undergoing maximal water diuresis is given pitressin in doses sufficient to concentrate the urine to a maximum, there

occurs no significant change in the rate of excretion of sodium. If urinary excretion reflects tubular reabsorption this indicates that provided the amount filtered at the glomerulus is unchanged, regardless of the concentration of the urine, the rate of sodium reabsorption may be virtually constant. Since the reabsorption of this ion largely determines the dilution of the urine and since this reabsorption may be unaffected during antidiuresis, a logical query concerns the relationship, functional and anatomical, between the diluting and concentrating operations becomes evident. Does the reabsorption of water without solute (T^{c}_{H2O}) take place through a segment of tubular epithelium which in the absence of ADH is almost completely impermeable to water yet so constituted that in the presence of this hormone it not only becomes permeable but is capable of promoting the movement of water against a considerable osmotic gradient. While conceding the possibility of such versatility, Smith considers it improbable and prefers to locate the concentrating site elsewhere in the nephron, functionally and anatomically discrete from the diluting segment, probably in the collecting ducts (45,46). Smith indicates that on clinical grounds one can suspect such independence: Loss of concentrating ability considerably antecedes loss of capacity for water diuresis in the progress of chronic renal disease, and in the recovery phase of acute renal insufficiency, the concentrating power is generally the last function to return (46). Experimental support of a more direct nature for this separation comes from the recent micropuncture studies of the rat kidney. Wirz (63) has successfully obtained fluid from the distal, as well as the proximal, tubule. The earlier finding by Walker et al (57) that proximal fluid is osotonic with plasma has been confirmed; this isotonicity obtains both during water diuresis and dehydration antidiuresis. In the first half of the distal convoluted

tubule, however, the fluid is invariably hypotonic, regardless of the ultimate concentration of the bladder urine. During water diuresis, this hypotonicity is maintained or increased along the course of the tubule. During antidiuresis, the fluid does become concentrated, attaining isotonicity by the middle of the distal convolution, but never exceeds isotonicity. Since bladder or ureteral urine examined simultaneously was considerably hypertonic to the plasma, this ultimate concentration must have been effected by abstraction of water, either without, or in considerable excess of solute further along the nephron, presumably in the collecting system. Wirz's observations have been confirmed by Gottschalk and Mylle (17) and together with Walker's earlier findings (57) afford rather solid support for the thesis, that concentration and dilution are functions of anatomically discrete loci within the nephron.

The geographic isolation of the concentrating locus having been established, the next problems concern the disposition of that fluid which enters the distal tubule before the concentrating segment is reached and the nature and site of action of the antidiuretic hormone. There being no direct observations on the action of this hormone upon the renal tubule, such information as is available derives inferentially largely from comparative physiology. The amphibian skin, which is under some control by the neurohypophyseal secretions, has long been recognised as an organ concerned with the over-all water balance of the organism. By the application of certain isotopic techniques to the study of isolated surviving segments of frog skin, Koefoed-Johnsen and Ussing concluded that the skin has the characteristics of a porous membrane, which, upon application of small amounts of posterior pituitary extract, behaves as if these pres had been opened

up (24). In other words, ADH had in some manner acted to increase the diameter of the pores so as to allow enhanced flow of fluid from one side of the membrane to the other. Analogising the functions of the kidney to that of the frog skin, the distal tubule of the foramen can also be conceived as a porous membrane, Under the influence of ADH these "pores" open, allowing water to diffuse out along the gradient created by the preceeding outflux of sodium until isotonicity is again established. Under the influence of ADH the waterproofing is in a sense removed and the free water permitted to diffuse out passively along its gradient.

Production of hypertonic urine, then, may be conceived as occurring in two phases. The first, an "isosmotic making" phase, is the passive diffusion of osmotically free water liberated by the active reabsorption of sodium, under direct control of ADH and serves to reduce the volume of fluid in the distal tubule, as well as to raise its concentration to that of the plasma. The second phase, the "hyperosmotic making" process, or T^{c}_{H2O} is the further reabsorption of an additional amount of pure water from this residual of isotonic fluid, rendering the tubular contents hypertonic to plasma. It is apparent that the movement of water during this latter operation must take place against an osmotic gradient: water is transported from an area of solute concentration, or low water activity, to an area of isotonicity, or relatively high water content. Since there seemed to be no reasonable alternative, this final abstraction of water necessary to produce hyperosmotic urine was thought to involve an active transport of water.

This hypothesis of Smith et al, was widely accepted and stimulated a great deal of work on clinical and physiological levels. The hypothesis however, contained the feature of active transport of water, and

this has always posed considerable conceptual problems. The favourite model for active transport processes generally consists of a biochemical pump which attacks molecules or ions of the transported substance one by one and which is driven by metabolism. In the case of ions, such a concept provides a reasonable working hypothesis. However, in the case of water, the situation is different simply because the rates of water movement by the hypothetical pump would exceed by several orders of magnitude the rates of oxygen consumption (8). Such extremely high rates of water movement occurring on a molecule by molecule basis raise virtually insuperable problems to any hypothesis explaining the link between the energy-yielding reactions of metabolism and the operation of the pump.

In 1951, a bold new theory was introduced to explain the mechanism whereby the kidney concentrates the urine. The theory stemmed jointly from Wirz, Hargitay, and Kuhn, (20, 65) all of the University of Basel. These investigators saw in the structural anrangement of the loop of Henle the possibility of the operation of a countercurrent multiplier system. It is interesting that the entire basis of the concept, together with a working model of the system had been published nearly ten years previously by Kuhn and Ryffel (26). This elegant proposal lay dormant because it seemed unnecessarily complicated in comparison with the accepted notion of urinary concentration by active reabsorption of water from the distal tubule and collecting ducts. Although recognition of the importance of a renal countercurrent system was fostered by the renewed activity in 1961 - of Kuhn and his collaborators, the general acceptance of such a scheme was promoted by the demonstration in 1955 by Brodsky et al (5) of the thermodynamic improbability of the theory of active water reabsorption. This group

concluded that the energy required to produce a concentrated urine by active water reabsorption is 1000 times the capability of the tubular cells. Clearly it was time to search for another mechanism to concentrate the urine; fortunately, the search was already under way.

The title of Kuhn and Ryffel's paper, published in 1942, may be translated "Production of Concentrated Solutions from dilute ones solely by membrane effects; a model of renal function" (26).

The authors described several countercurrent systems by which it was possible to produce concentrated solutions from dilute solutions (a large effect) by multiplying small, single effects. They drew the attention to the features these countercurrent systems had in common, with the anatomical arrangement within the kidney of Henle's loops, vasae rectae and collecting ducts, and suggested that Henle's loops act as countercurrent systems.

The basic feature of a countercurrent system is that two streams of fluid moving in opposite directions become so juxtaposed as to exchange energy or material in accordance with the forces acting upon them. The renal medulla has a unique spatial configuration as a result of the anatomical orientation of the structures within, which provides a virtual battery of countercurrent systems arranged in parallel (Diagram 3). The ascending and descending limbs of the loops of Henle constitute one such system, and the arterial (descending) and venous (ascending) limbs of the vasae rectae, intermingling with and parallel to the loops of Henle, form another. Hargitay and Kuhn (20) formulated the theory that the mechanism of concentration of the urine is based on the presence of a longish tube that bends back on itself, so that flow in the two limbs is opposite in direction (hairpin countercurrent), combined with some active process that

creates a small concentration difference between the two limbs, the descending being slightly hypertonic relative to the ascending. The multiplier effect begins to operate when the relatively hypertonic contents of the descending limb are pushed around the hairpin bend by bulk flow: the ends of the two limbs near the bend are now in osmotic equilibrum, and the slight pressure difference between them is temporarily abolished. If the same active process continues to operate, maintaining a small osmotic gradient across the boundary between the two compartments, the fluid in the lower portion of the descending limb then becomes even more hypertonic than it was at the beginning. This still more concentrated solution once again flows around the bend, and at the lower end of the system the two limbs re-equilibrate at an osmotic pressure higher than that which initially obtained. Continuous action of such a mechanism will ultimately result in a steady state: fluid within the entire system will become progressively more concentrated towards the hairpin bend and rediluted on its way back up the ascending limb, resulting in an osmotic stratification along the long axis of Henle's loop, whereas the concentration difference between the limbs, at any given level, will be no greater than that created by the initiating active process. Osmotic pressure many times higher than the initiating single effect may be produced. The result of the countercurrent mechanism in the loops would be a milieu (the adjacent interstitial fluid of the renal medulla) of increasing hypertonicity towards the tip of the renal papilla. Concentration of the final urine was then conceived to occur in the collecting ducts through a passive withdrawal of water from the fluid in them as they traverse the progressively more hypertonic medullary interstitial fluid. That the final concentration of the urine occurs in the collecting ducts is in accord with the classical theory of Smith: the disagreement

concerns the mechanism by which water is removed and in the role played by the loop of Henle.

To fulfil the requirements of this hypothesis it should be able to demonstrate that in a loop of Henle: (1) The osmotic pressure at each level perpendicular to the axis of the system is the same. (2) The osmotic pressure increases from the base to the tip of the system, i.e. from the cortex to the tip of the papilla. (3) The osmotic pressure of the fluid leaving the system is hypotonic with respect to the osmotic pressure of the fluid flowing into the system.

Evidence that the first of these conditions actually obtains within the kidney was presented in 1951 by Wirz, Hargitay and Kuhn (65). These workers showed by microcrysoscopy on 30 μ-thick tissue slices, cut perpendicularly to the axis of the papilla, that the osmotic pressure of the content of cortical tubules equals the osmotic pressure of the blood, and further that the osmotic pressure in the medullary tubules increases continuously to the tip of the papilla. They showed, moreover, that at any one level, no differences were discernible between the tubular urine whether it is in the ascending or descending limbs of the loop of Henle, the capillary blood, and the urine in the collecting ducts. This approach revealed the kidney to be composed of concentric shells, each of uniform osmotic concentration. The outer shell consisted of the cortex and was isosmotic with the peripheral plasma. Each concentric inner shell in the medulla, however, had a higher osmolality than the one outside it, with the innermost, smallest shell at the papillary tip having the highest osmolality of all. These findings were recently confirmed by Bray (4).

Another valuable approach has been afforded by the analysis of cortical and medullary slices for specific solutes. That the chloride content of medullary tissue in the rabbit increases from the outer medullary zone to the tip of the papilla has been known for many years (16, 34). More recently Ullrich and Co-workers (50,53) have shown in hydropenic dogs that the concentrations of sodium, urea, exogenous creatinine and aminoacids increase progressively, in a similar manner, though not all to the same extent, from the cortex to the tip of the papilla. These observations have been confirmed for sodium in studies using Na^{22} in the rat kidney (25) and by direct analysis of sodium, chloride and urea in the dog kidney (31,32). The rapid accumulation and the ultimate axial gradient of I^{131} tagged albumin in the dog medulla (28, 33) conforms with this pattern. A further piece of supporting evidence is thefinding of Ullrich and Jarausch (53) of a good linear relationship occurring between sodium concentration in the tip of the renal papilla and the final osmolality of the urine. This would be expected if the hypertonicity of the renal medullary fluids is due to an accumulation of sodium and chloride.

A third line of evidence implicating the countercurrent system consists of measurement of osmolality of fluid obtained by micropuncture. In 1953, Wirz (62) showed that the renal papillary blood, collected by micropuncture of the superficial capillaries of the papilla in the golden hamster is essentially isosmotic with the bladder urine, as is required by the counter-current theory. This has been amply confirmed (17,56). However, information on the tubular urine was until recently confined to the convolutions near the surface of the cortex. Wirz, in 1956 (63) demonstrated by micropuncture that fluid obtained from the first two thirds of the proximal tubule was isosmotic with the peripheral plasma and that the fluid

leaving the loop of Henle is hyposmotic. These findings, first noted in the early micropuncture studies of Walker et al (57) has since been confirmed by Gottschalk and Mylle (17).

At the time (1951) when Hargitay and Kuhn (20) published their paper on the countercurrent theory, and Wirz, Hargitay and Kuhn (65) on the kidney, nothing was known about the composition of the urine between the proximal convoluted tubule and the renal pelvis, and the physiological characteristics of the loop were not spelled out. It was poisted that the "single" or initial effect, or "vis a tergo" of the countercurrent system in the medulla consisted either of the movement of water from the descending into the ascending limb (which virtually amounts to active water transport) or conversely of salt (sodium chloride) from the ascending to the descending limb; they failed, however, to recognise that the latter mechanism could not operate as an osmotic multiplier unless the permeability of the ascending limb to water is restricted.

After Wirz in 1956 had demonstrated the osmotically dilute nature of the urine in the early distal convolution in hydropenic animals, (63) he recognised that the ascending limb of the loop must be relatively impermeable to water and he favoured the belief that this dilution was effected by the reabsorption of sodium chloride in this segment (he did not distinguish between the ascending thin and thick segments). The permeability characteristics of the descending limb were not specified, though it was clear that sodium chloride must gain access to (and probably water lost from) this limb if the system is to work as a countercurrent multiplier. As in the original paper, it was poisted that the progressive accumulation of sodium chloride along the long axis of the loop increased the osmotic

pressure of the interstitium and concentrated the urine by passive abstraction of water through the collecting ducts (63,64).

Recognising the theoretical difficulties of the 1951 description, and discounting Wirz's necessarily tentative suggestions of 1956, Berliner and his colleagues in 1958 (3) proposed that sodium chloride is actively reabsorbed throughout the length (descending and ascending limbs) of the thin segment, and, as a necessary corollary, that this segment is everywhere essentially impermeable to water. Here, the loop does not operate as a countercurrent mechanism in any case, though the vasae rectae continue to serve as countercurrent exchanges. In this hypothesis, however, the urine at all points around the loop should be hypo-osmotic to the blood in consequence to the removal of sodium. The recent and contrary demonstration by Gottschalk and Mylle (17) that the urine at the tip of the loop is actually isosmotic with the urine in the collecting ducts completely negates this hypothesis.

The interpretation proposed by Gottschalk and Mylle (17) which fits the facts recited to this point, is that sodium chloride reabsorption begins in the ascending limb of the thin segment and continues in the thick segment of the ascending limb and throughout the distal convolution. The entire ascending limb (thin and thick) is conceived to be relatively impermeable to water, thus assuring delivery of a dilute urine to the distal convolution. These workers demonstrated that infusion of sodium chloride increased the hypotonicity of the distal urine and therefore presented a strong evidence that the loop does in fact reabsorb sodium at some more proximal region, and that it operates on sodium rather than on water per se. As a working assumption, the descending thin segment is conceived to be permeable to

water (and possibly sodium chloride) as was implied by Wirz (63); by this postulate the descending urine will come into osmotic (and sodium ?) equilibrium with the interstitium, the system will serve as a true countercurrent multiplier, and the osmotic pressure of the interstitium will increase along the long axis of the loop to reach a maximal value at the tip, or the "reversal point", which may be taken to be the point where the water and sodium permeable descending limb, gives way to water impermeability and active sodium reabsorption in the ascending limb. Again, passive diffusion of water out of the collecting ducts serves to concentrate the urine, and the vasae rectae, being in free interchange with the interstitium, serve as countercurrent exchangers, promoting the efficiency of the system and carrying away the sodium chloride reabsorbed locally and the water abstracted from the urine.

This is essentially the hypothesis formed by Wirz in 1956 (63,64) but now spelled out in more detail and supported by direct analysis of the urine at the tip of the loop of Henle - the all important datum required to substantiate a countercurrent hypothesis. Neither here nor in previous theories is there any requirement that the tubular or vascular channels be physiologically juxtaposed because lateral diffusion over microscopic differences can effect sodium and water equilibration.

But the problem may be more,or less,complicated than this. Hilger, Klumper and Ullrich (21) have catheterised the collecting ducts with small polyethylene catheters and found that the urine became concentrated by water reabsorption passing through the collecting ducts. Moreover, not only water, but solutes too, are reabsorbed from the collecting ducts. A minute analysis showed that chiefly sodium ion andurea left the collecting

ducts (23). A large part of the reabsorbed sodium ion is exchanged against hydrogen ions and ammonia (51,52). These and other observations (55) led these investigators to dismiss the thin segment as playing only a passive role in the concentrating mechanism - in effect, they treated it as freely permeable to water, sodium chloride, urea and presumably other solutes in the interstitium. They suggested that the "initial concentrating effect" in the concentrating operation is the reabsorption of sodium chloride in the thick segment of the ascending limb (outer medulla) whereby the urine is diluted and the interstitium is enriched osmotically. Sodium reabsorption by the collecting ducts also enriches the sodium content of the inner medulla, a process which they called the "terminal concentrating effect". These two operations, they believed, suffice to concentrate the urine.

This interpretation has the advantage of shifting active sodium reabsorption out of the thin segment but it presents several difficulties. If we assume that, at any specified level, the urine and interstitium are approximately in osmotic equilibrium, relatively more sodium than water must be removed from the urine in the collecting ducts if the interstitial osmotic concentration is to be increased, and this operation alone would serve to dilute rather than concentrate the urine inside the duct.

The location of the sodium pump in the thick ascending limb of Henle's loop as Ullrich et al at first believed - and contrary to Wirz's original hypothesis which considered that active sodium transport occurred over the entire extent of the ascending limb of Henle's loop, is very attractive and agrees well with the cytological characteristics of the lining epithelium of these two segments of the ascending limb. But the recent studies of

Gottschalk et al (19), however, clearly show that osmotic pressure continues to rise in the inner zone of the medulla. This was later confirmed by Ullrich himself and his co-workers (56). Also Schmidt-Nielsen and O'Dell (41) have recently demonstrated the same finding in different animals with varying lengths of the thin segment in the ascending limb of the loop and concluded that the sodium pump which has been demonstrated in the thick ascending limb of the loop of Henle must function in a similar manner in the thin ascending limb.

A special role for urea in the concentrating process has been explored by Berliner et al (3), Schmidt-Nielsen (40) and Klumper, Ullrich and Hilger (23). Urea, despite its apparently free diffusibility within the organism in general, is present in the urine at concentrations much higher than its plasma level, a finding which indicates relative impermeability of the nephron to this substance. Ullrich and Jaransch (53) found that of all the substances measured (Na, K, Cl, Ma, Ca, Inorganic phosphate urea, aminoacids, and exogenous creatinine), the highest concentration gradient was achieved by urea, the concentration of which at the papilla nearly equalled that in the urine. Berliner et al (3), implying that the collecting duct is particularly permeable to this substance, as compared with the rest of the nephron, suggest that urea diffuses out of the duct along with water and is deposited in the medullary interstitium along with the sodium already present. The urine thus contributes some of its urea to the osmotic stratification, enabling the establishment of a higher peritubular osmotic concentration than can be effected by the salts of sodium alone. The function of urea within the interstices is thus to balance to a large extent the osmotic effect of the

urea within the collecting tubule, countering any restraining effect of the latter upon the outward diffusion of water. This has the extraordinary consequence that correspondingly less osmotic work is required for the excretion of urea, and a given osmotic load of urea entails a correspondingly smaller excretion of water in the urine than does an equal osmotic load of sodium chloride, mannitol, etc. to which the collecting ducts are impermeable (49).

A different explanation has been offered by Schmidt-Nielsen (40) who suggests that the high medullary concentration of urea is not the result of simple passive diffusion. This author proposes an active tubular transport of urea against a concentration gradient; this small process is in turn amplified by the multiplier effect of countercurrent flow within the medullary structures, resulting in a high interstitial, and, consequently, a high urinary concentration of this substance.

Three attractive features of the countercurrent hypothesis have been noted by all investigators. First is the fact that the urine is concentrated by an osmotic gradient down the long axis of the loop, with only a small gradient across any one cell layer in the lppp. Second, the hypothesis dispenses at long last with the active transport of water molecules - the entire operation is mediated by the active transport of sodium chloride, a process that to one degree or another is going on throughout the length of the nephron. The third attractive feature is that, for the same mechanism (sodium chloride reabsorption) to serve either to concentrate or to dilute the urine, only a redefinition of the locus of action

of the antidiuretic hormone may be required.

It is widely accepted that the action of ADH is "permissive", in that it simply promotes passive osmotic equilibration between the tubular urine and the blood by increasing the permeability of the "tubular" epithelium to water (49). Sawyer (39) and Wirz (64) have independently attributed this action to the opening of hypothetical "pores" which facilitate the diffusion of water, in a manner analogous to the action of the neurohypophyseal water-balance principle on amphibian skin (24). Without specific reference to this "pore" theory, Ginetsinskii et al (12, 14,15) have suggested that ADH liberates hyaluronidase in the tubules and that this enzyme depolymerises the hyaluronic acid cement between the epithelial cells and lets water pass through.

It has been noted that during antidiuresis, osmotic equilibration between urine and blood is attained from the middle of the distal convoluted tubule on the urine remaining isosmotic until it enters the collecting duct. In water diuresis, however, the urine remains dilute, throughout the distal convolution, and, in fact, it appears to be further diluted between the end of the distal tubule and the bladder (17, 64).

The simplest interpretation of these data is to suppose that the locus of (the "pore") action of ADH extends from the early part of the distal convoluted tubule all the way down the collecting ducts (3, 17, 21, 39, 64). Hence, in the absence of ADH, all)or nearly all) the osmotically free water generated by the reabsorption of solutes (mainly sodium chloride) in the loop of Henle and the distal segment remains to be delivered to the collecting ducts; since these are relatively impermeable to water in the absence of ADH and therefore isolated from the hyperosmotic

medulla, this free water emerges from the kidney as a copious volume of dilute urine. No direct proof has been presented by the various workers for this assumption on the site and mode of action of ADH. Ginetsinskii, however, claimed that he could demonstrate, by light microscopy, disappearance of the mucopolysaccharide intercellular streaks in the collecting tubules at the height of antidiuresis, and considered that this indicates opening up of intercellular "pores" for the reabsorption of water (12,13).

This review of the ideas and theories of the mechanisms of urinary concentration and dilution has drawn the attention to some important facts. First, it has shown that although the principle of countercurrent flow was first applied to the kidney in 1951, it has been regrettably neglected until recently. It can, therefore, be looked upon as a "new" theory, and novelty tempts speculation. Secondly, it is all too apparent that much of present day hypothesising about the mechanisms ultimately responsible for dilution and concentration of the urine is largely conjectural; fabrication has been out of cloth generously perforated by large gaps of knowledge. The secrets of the characteristics of the tubular membranes and their metabolic activities, for instance, remain largely to be discovered. Thirdly, the idea that the squamous epithelium of the thin segment of the loop of Henle is freely permeable to water (if not to sodium also) in the descending limb, and suddenly acquires water impermeability and active sodium transport at the tip of the loop for no better reason, apparently than the circumstance that it has turned a corner,is extravagant, physiolgically complicated and difficult to believe.

What does emerge from the mass data presently at hand is that (1) the hairpin configuration of the blood vessels and loops of Henle is not an

anatomical quirk but implies specific functional significance with respect to the excretion of water. (2) Osmotic stratification within the renal medulla seems to be definitely established. (3) This osmotic stratification is due to a countercurrent multiplier effect by the loops of Henle and a countercurrent exchanger function by the vasae rectae.

Many questions are still to be answered. Does the sodium chloride, pumped out from the lumen of the relatively water-impermeable ascending limb into the medullary interstitium, induce osmotic withdrawal of water from the water permeable descending limb (17,64), or whether sodium chloride is pumped out from both the descending and ascending limbs, the differentiation between the two limbs being due to a high water permeability of the descending and low water permeability of the ascending limb creating an osmotic difference between them at any level or whether, alternatively, the descending limb is also relatively impermeable to water and it transports sodium inwardly by an active transport mechanism (61). The second important question is whether ADH really acts on the distal convoluted and collecting tubules, as has been suggested by all the authors without any real evidence, apart from it being the simplest interpretation of the accumulated data. Thirdly, how can the tubules vary their degree of permeability to water and/or to solutes in the different functional states; does this involve cellular, intercellular or extracellular alterations?

Renal physiologists seem to have largely exhausted their methods and special techniques of investigation of this problem and they are unlikely to get much more data, by the now familiar methods of microcrysoscopy and microanalysis of thin renal slices, or by micropuncture of the renal tubules and capillaries, however clever and accurate these techniques are. Micro

analysis of the chemical composition of a slice of renal tissue several microns thick, though very valuable in giving an overall picture at this particular level in the kidney, can never discriminate between minute differences in the composition of fluids in the descending or ascending limbs of the loop, the collecting tubules, the interstitium and the capillaries; and these are exactly the data required at this stage to fill the gaps in our knowledge. Similarly, many parts of the renal tubule are not accessible to micropuncture, particularly the tubules in the outer medullary zone(the all important zone in the countercurrent hypothesis); the pars recta of the proximal tubule, the thick segment of the ascending limb of the loop of Henle and the intermediate part of the collecting tubules. The pars recta of the proximal tubule, for instance, plunges deep into the kidney and differs morphologically, and possibly functionally, from the pars convoluta on the kidney surface. Possibly, countercurrent diffusion occurs here as in other parts of the loop of Henle. It is quite hazardous, therefore, to extrapolate the water reabsorption curve, as Walker et al. have done (57), beyond the experimentally determined points, and the water content, inulin, sodium chloride and urea concentrations at the very end of the proximal tubule should remain a matter of speculation.

I intended by this, rather detailed, introduction to the subject to focus the attention to the fact that a new method of approach to this problem, from an entirely different angle, is needed, if light is to be quickly thrown on the still unanswered questions. One of the oldest methods adopted by biologists and research workers in the basic, fundamental medical sciences, is a correlation of physiology to morphology. However, bored with the type of descriptive morphology - with its attendent artefacts -

available by light microscopy, biologists have been directing their attention more and more to the biochemical study of tissues. This would have been an admirable thing, had not the pendulum swung much too far in the opposite direction so that many workers were almost totally ignorant and even secretly contemptuous of the fundamental structure of the cells and tissues with which they were concerned. To them, the cell was a small ampoule of assorted chemicals which had to be cracked open so that its contents might be suitably examined in the test tube. This resulted in a pernicious and unnatural divorce between morphology and physiology. However, within the last few years, encouraging signs became apparent and the pendulum is swinging back. Biochemists are becoming increasingly interested in the intact cell and all its multifarious activities. They are also beginning to appreciate the difficulties of interpretation experienced by those who study tissues with the microscope and who see the immense complexity of structure of many organs - a complexity often so great, in fact, that it is difficult to understand how the chemical analysis of crude samples, however small, could yield much of value.

But, of course, at the molecular level attained by electron microscopy, chemistry and morphology become one, and the histologist and the chemist meet on common ground. It is by the electron microscope that I intended to study the mechanism of dilution and concentration of the urine and the site and mode of action of the antidiuretic hormone in the mammalian kidney.

Material and Methods.

16 albino rats, "Wistar" strain between 250 and 400 gm. in weight, were used for this study and were divided into the following groups:

I. Hydration Experiment:

Six rats were forcibly hydrated by the administration of water through an intragastric tube. Three were given 5 ml. each and the other three were given 20 ml. each in a single dose. The rats were stimulated to pass urine at 15 minute intervals and the urine samples were measured for volume and osmolality. The osmolality was measured by a Fiske Osmometer, based on the determination of the depression in the freezing point, using the small head adaptor. The rats were killed at the height of the diuresis, when the urine osmolality was lowest.

II. Hydration followed by dehydration experiment:

One rat was given 5 ml. and two other ones 20 ml. water each by an intragastric tube. The rats were then placed separately in individual metabolism cages. Solid "pellet" diet was allowed but no water was given. The rats were killed after 50 hours of dehydration, the osmolality of the urine passed within the last two hours before death was measured.

III. Dehydration experiment:

Four rats were used in this experiment, one kept without water for 24 hours and the other three for 48 hours. The first two rats were also kept without food while the latter two were allowed solid "pellet" diet. The osmolality of theurine passed within the last two hours was determined.

IV. Pitressin experiment:

Each of three rats was given 50 milli-units aqueous Pitressin (Vasopressin injection, B.P., Parke, Davis & Co.) in the tail vein. They were stimulated to pass urine at 15 minute intervals and the urine was measured for volume and osmolality. They were killed when the urine passed showed an osmolality of above 2,000.

After killing the rats one kidney was fixed in corrosive formol and used for light microscopy, while blocks from the other were fixed for one hour in 1% buffered osmium tetroxide, embedded in methacrylate and araldite and examined by the electron microscope, as described previously. Again, particular care was taken to choose blocks for electron microscopy from the superficial cortex, the outer zone of the medulla and the papilla.

Results.

I. Hydration Experiment:

The urine osmolality at the time of attaining maximum diuresis is seen in Table (5).

Table 5.

Rat No.	Weight g.	Volume of water administered ml.	Time of maximum diuresis after administration minutes.	Urine Osmolality m.Osm/Kg.
1	370	5	60	120
2	380	5	90	400
3	370	5	90	330
4	400	20	90	73
5	250	20	120	199
6	260	20	120	100

On light microscopy: no abnormality was detected.

On Electron microscopy:

Glomerulus: A slight degree of dilatation of the capillaries was observed

in all the glomeruli examined. The dilated capillaries contained few erythrocytes. The endothelial fenestrae appeared slightly wider than normal. The dilatation was more marked in the rats that received 20 ml. of water. This dilatation can be easily explained by the rapid increase in the blood volume, the sharp rise in the renal blood flow and the increased glomerular filtration rate which follow such a large single dose of water.

In some glomeruli, the epithelial cells showed peculiar "worm-like" structures, consisting of a double membrane, in their cytoplasm (Fig. 157). These cells were otherwise entirely normal.

Proximal tubule:

A. Pars convoluta: The proximal convoluted tubules were dilated; some of them only slightly so that a lumen just begins to appear in the centre of the honeycomb of microvilli (Fig. 158), while others, were markedly dilated (Fig. 159). Many of these tubules showed cells in the process of being shed off into the lumen (Fig. 159); a process which can be seen only very occasionally under normal conditions, was seen rather frequently in these hydrated animals. This resulted in the appearance of the lumina of some tubules to be filled up by cellular debris (Fig. 159). Otherwise, the cells of the proximal convoluted tubules were quite normal. Particular attention should be drawn to the basement membrane of these tubules. It was normally thin in all the superficial tubules (Fig. 158) but as more deeply situated proximal tubules were examined, some of them began to show a thickening of the basement membrane, particularly the very dilated ones (Fig. 159), while most of them still possessed a normally thin basement membrane (Fig. 160). In the deep cortex, on the other hand, more

proximal tubules had a thick basement membrane, which sometimes was very thick indeed (Fig. 161), measuring more than 10 times the normal thickness. This thick basement membrane gave the impression of being a soft gel, with the basal cell foldings pushing through it and embedded in its superficial part (Fig. 161).

B. Pars recta: Most of the proximal tubules in the outer medullary zone were dilated (Fig. 162) and the process of shedding off of lining cells into the lumen was again rather frequently encountered (Fig. 162). The cellular cytoplasm showed an abundance of the vacuoles which are normally present. Both types of vacuoles were numerous, particularly, the vacuoles with the clear contents (Fig. 162). They were not restricted to the apical, relatively clear, part of the cell, though they were more numerous there.

The most striking change in this part of the nephron was found in the basement membrane. Practically all the tubules had a very thick basement membrane,(Fig. 163) , and the basal cell foldings markedly indented this thick membrane and in some places gave the impression of being buried in it (Fig. 164 and 165). The basement membrane measured between 1 - 1.5 μ (the normal thickness is about 0.1 μ) and was frequently uniform in appearance. In some tubules, however, the basement membrane was seen to split in one particular point and to contain a granular osmkophilic deposit in between its layers (Fig. 166) while in others, the greater part of the circumference of the tubule was surrounded by a thick fibrillar, laminated basement membrane (Fig. 167).

Thin segment.

A. In the outer zone of the medulla -

All the thin segments in this zone (all are on the descending limb of the loop of Henle) had a thick, fibrillar basement membrane, which measured 0.3 - 0.5 μ as contrasted with the normal thickness of 0.1 μ (Fig. 168). This basement membrane change was noticed in all the hydrated rats, whether they received 5 ml. or 20 ml. water.

In the rats that received 20 ml. of water, an additional change was sometimes noticed in the thin segments in this zone. Some individual cells were markedly swollen, their cytoplasm rarified and they looked as if they have imbibed a large amount of water (Fig. 169). The basement membrane of these cells was not as thickened as the remainder of it surrounding the rest of the tubule. However, particularly in the rats that received the large volume of water, there appeared to be a simplification, flattening or ironing out of the basal cell foldings and the cells appeared to have a rather sparse cytoplasm (Fig. 170) and this may be the early stage of the extreme swelling occasionally seen in some individual cells.

B. In the inner zone of the medulla -

The thin segments in this zone showed various significant changes. About half the total number of thin segments examined, showed changes which were quite different from these shown by the other half.

I) Half the thin segments showed thickening and fibrillation of their basement membrane, in exactly the same manner as seen in the thin segments in the outer medulla (Fig. 171,172,173). In these tubules, the simplification, flattening or ironing out of the basal cell foldings as well as the sparse appearance of the cellular cytoplasm, was also quite similar to that observed in the outer medullary thin segments. Similarly, the marked swelling of individual cells was again observed (Fig. 174). Some

tubules had wide bare areas with only the basement membrane separating the lumen from the interstitial space (Fig. 175). This latter change was particularly encountered in the rats that received the larger volume of water. This change apparently resulted from the disappearance of individual cells that might have exploded after being extremely swollen. II) The other half of the thin segments showed quite different changes. Their basement membrane was normally thin (Fig. 176). The luminal surface of the cells was thrown into a number of folds and the cells sometimes showed papilliferous processes into the lumen (Fig. 177). The cell cytoplasm contained a large number of vacuoles with clear contents. These vacuoles were frequently intimately related to mitochondria (Fig. 178). In these thin segments, flattening of the basal cell foldings was also observed. In the rats that received 20 ml. water, some cells in these tubules were seen in a process of separation from their thin basement membrane (Fig. 178 and 179), eventually also leaving bare areas of basement membrane.

The fact that only half of the thin segments in the inner medulla are descending while the other half are ascending together with the fact that the morphological changes observed in half the inner medullary thin segments differ from those observed in the other half, seem to indicate that the behaviour of the descending thin limb of the loop of Henle differs from that of the ascending thin limb in response to acute hydration. Since the thin segments in the outer zone of the medulla are all descending, and since the changes described in I) in half the inner medullary thin segments are similar to those observed in the outer medullary thin segments, it is concluded that the basement membrane of the tubules is only thickened if they are descending, but remains normally thin if they are ascending.

Thick segment:

The thick segments always appeared quite normal. In particular, their basement membrane was normally thin (Fig. 180) as contrasted with that of the pars recta of the proximal tubule (Fig. 164).

Distal Convoluted Tubule:

No change was seen in any distal convoluted tubule.

Collecting Tubule:

A. The dark cells: A peculiar change was observed in the dark cells of the collecting tubules, which was equally apparent after the small as well as after the large dose of water. The cells appeared very active. Their basal cell foldings increased in number and in depth, the luminal surface became thrown into a large number of folds and they "grew" papilliferous processes, the surface of which was thrown into a number of microvilli (Fig. 181).

B. The light cells: After 5 ml. water, many clear vacuoles appeared within the cell cytoplasm in the light cells of the collecting tubules particularly in the inner medullary zone (Fig. 182). Some of these vacuoles could be traced as they passed between the cell membranes of two adjacent cells (Fig. 183), or between the cell membrane and the basement membrane within the basal cell foldings (Fig. 184), and sometimes even in the interstitial space adjacent to the wall of a thin fenestrated (efferent) capillary (Fig. 185). Some of these vacuoles had a crescentic dense structureless body at the periphery (Fig. 186 and 187) or a small densely osmiophilic body in the centre (Fig. 188). These granulated vacuoles were frequently closely related to the mitochondria of the cells, and a transition from a normal mitochondrion with cristae in which a dense body appears at one side

to a large dense granule in which all mitochondrial internal structure has been obliterated, to a dense granule with a small vacuole and finally to a vacuole with a small granule, could be traced (Fig. 189).

After 20 ml. water, although the light cells showed vacuoles and dense structureless granules in their cytoplasm particularly in the inner medullary zone, the most striking change noticed was lateral separation of cells. Lateral separation appeared to begin on the basement membrane side of the cells, while they remained adherent at the terminal bar (Fig. 190). The degree of lateral separation was noticed as it gradually increased (Fig. 191 and 192) until finally, the terminal bar gave way (Fig. 193). This resulted in a tubule incompletely lined by cells with bare areas of basement membrane only separating the luminal contents from the interstitial space (Fig. 194 and 195) and in some tubules, extensive stretches of basement membrane were completely bare (Fig. 196).

The collecting tubules appeared to be slightly dilated but their basement membrane was absolutely normal in appearance.

Capillaries:

A. In the cortex: The intertubular capillaries were quite normal.

B. In the outer zone of the medulla: The thick type of capillary (the afferent or descending vasae rectae) showed thickening of their basement membrane to twice or three times the normal thickness (Fig. 197 and 198). This was particularly marked in the inner stripe of the outer zone of the medulla (Fig. 199). The thin type of capillary (the efferent or ascending vasae rectae) had a normally thin basement membrane (Fig. 200).

C. In the inner zone of the medulla: Many capillary endothelial cells were markedly swollen mainly because their cytoplasm was full of a large number

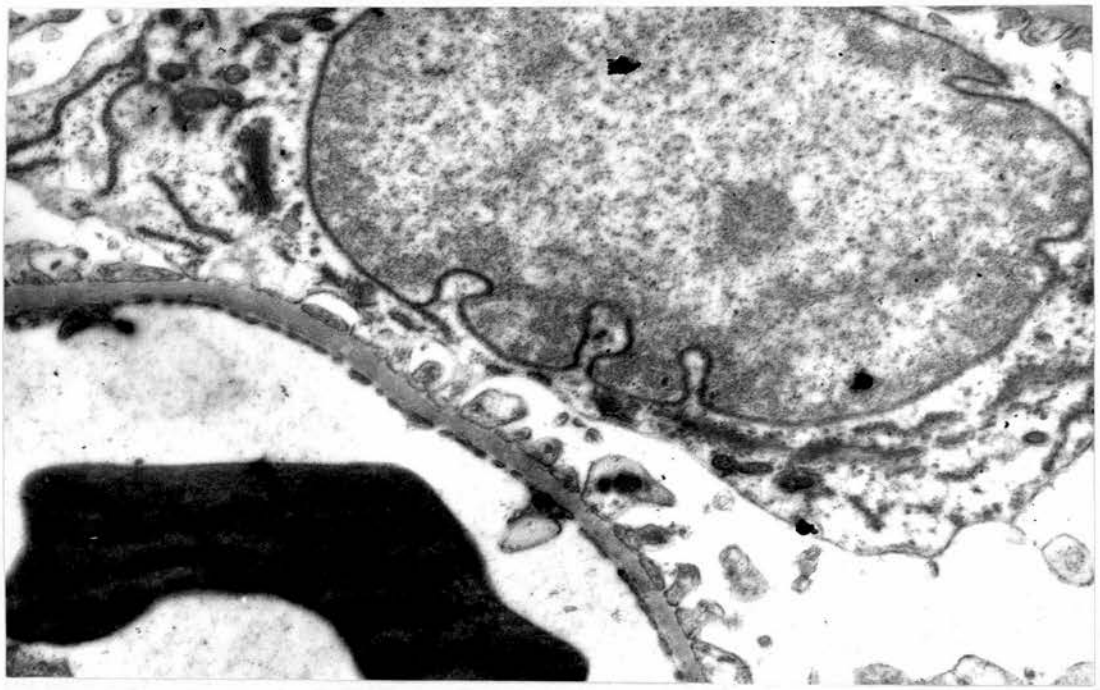


Fig. 157. Hydration experiment: Rat 4. Glomerular epithelial cell. Note the string-like bodies in the cytoplasm. x 15,000

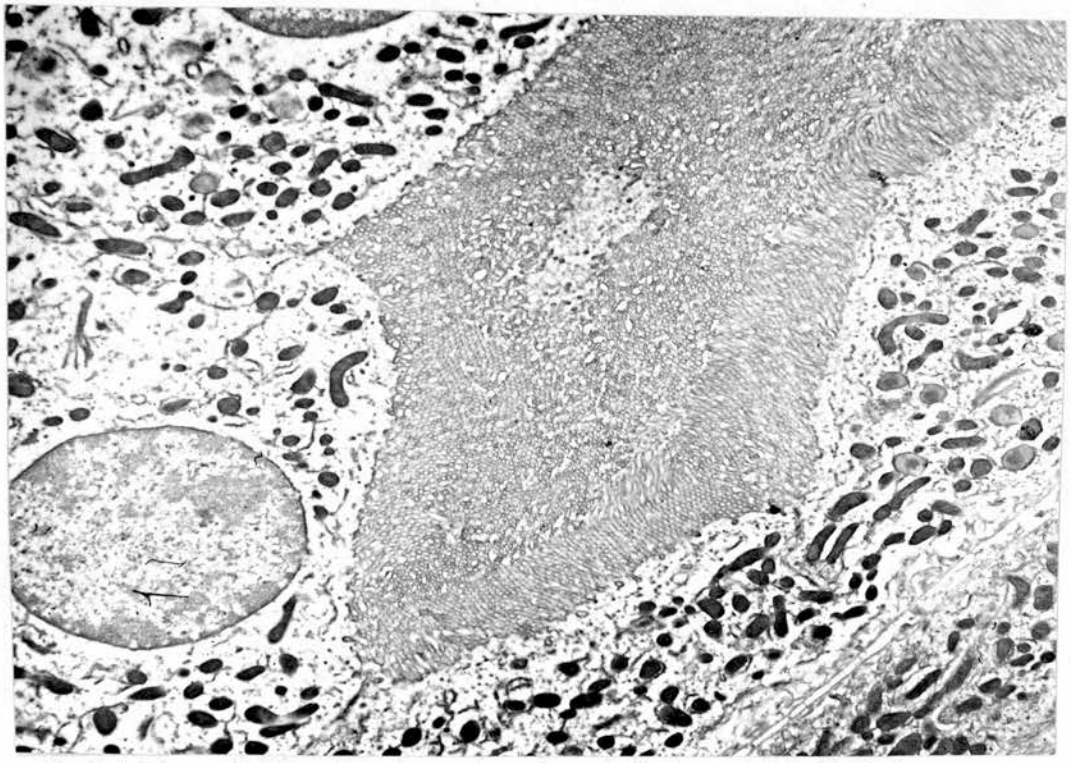


Fig. 158. Hydration experiment: Rat 4. Superficial cortical proximal convoluted tubule. Note the thin basement membrane and the lumen appearing within the packed microvilli. x 4,000

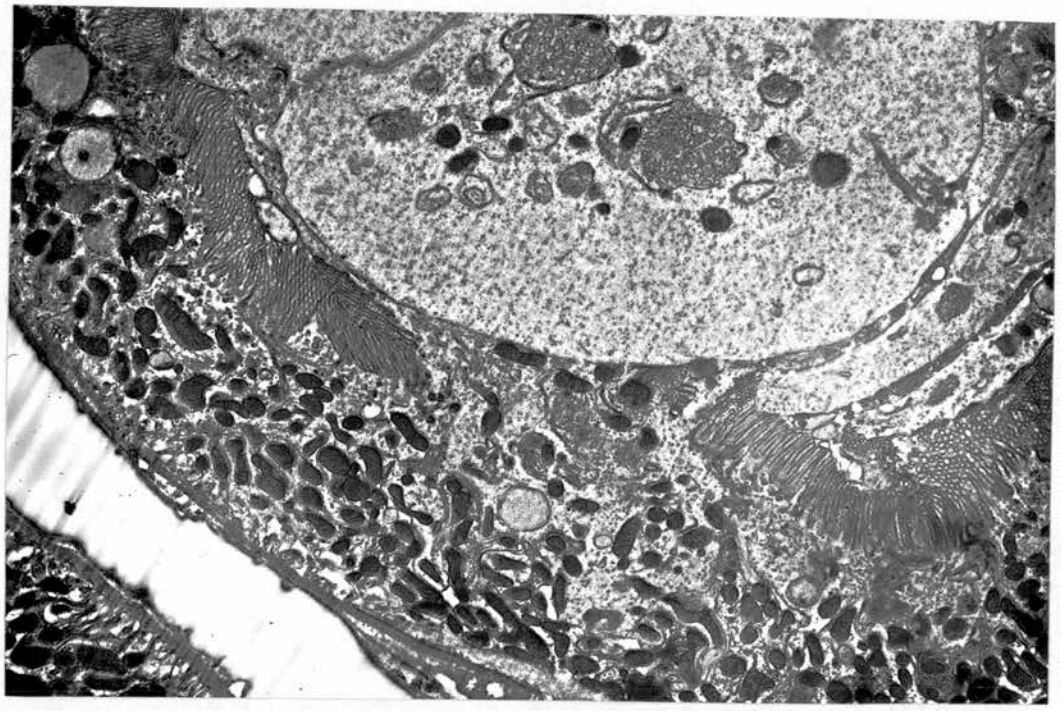


Fig. 159. Hydration experiment: Rat 4. Deep cortical proximal tubule. The basement membrane is slightly thickened. The lumen is very dilated and is full of cellular debris and one cell is apparently in the process of being shed off into the lumen. x 3,000

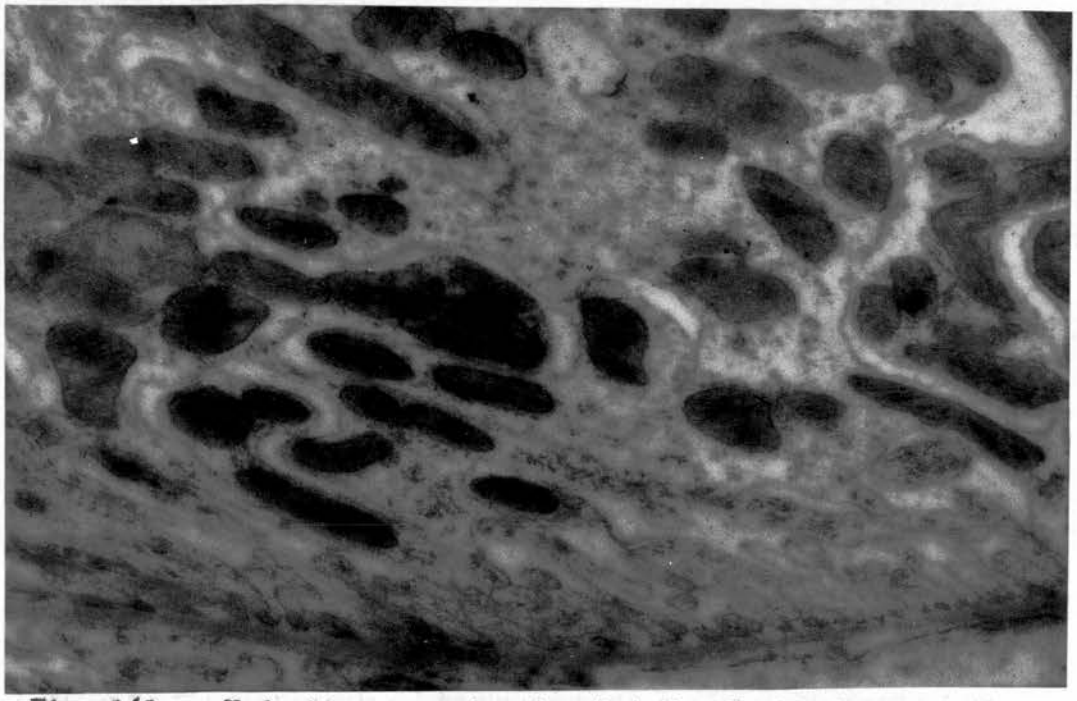


Fig. 160. Hydration experiment: Rat 2. Cortical proximal convoluted tubule. The basement membrane is of normal thickness. x 27,000

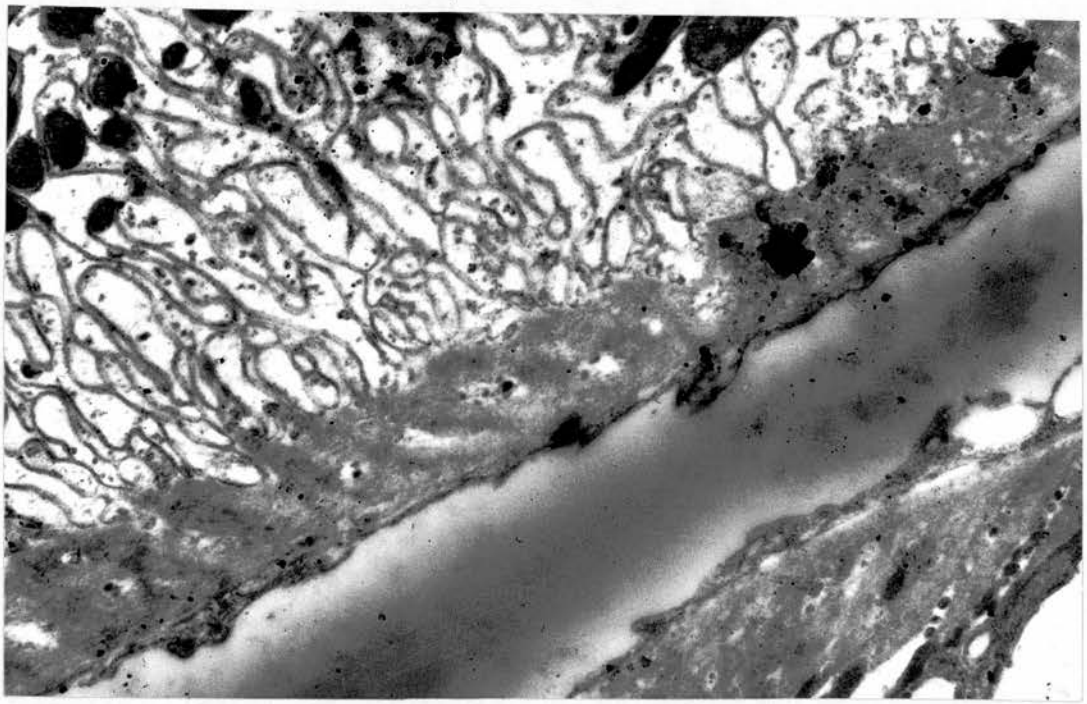


Fig. 161. Hydration experiment: Rat 5. Two deep cortical proximal tubules. The basement membrane is very markedly thickened measuring 1.25 μ in thickness. Note the basal cell foldings as they dip into thin swollen, apparently jelly-like basement membrane. x 15,000

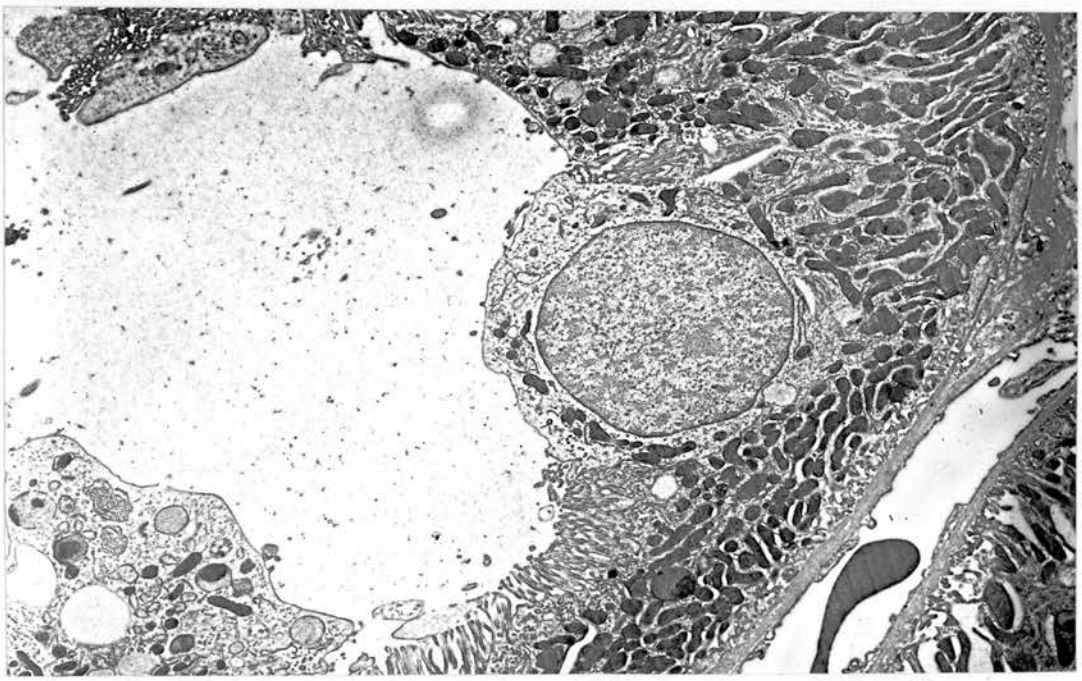


Fig. 162. Hydration experiment: Rat 5. Pars recta of a proximal tubule in the outer medullary zone. The lumen is very dilated and the basement membrane is thickened and is seen to split up in a fibrillar fashion for a short distance on the right side. Note that two cells are being shed off into the lumen and that the cytoplasm is full of vacuoles with clear and vacuoles with granular content. x 2,500

Fig. 163. Hydration experiment: Rat 6. Pars recta of a proximal tubule. Note the marked thickening of the basement membrane. On the right side it appears serrated and the basal cell foldings can be seen as if they were pushing into it.
x 2,500

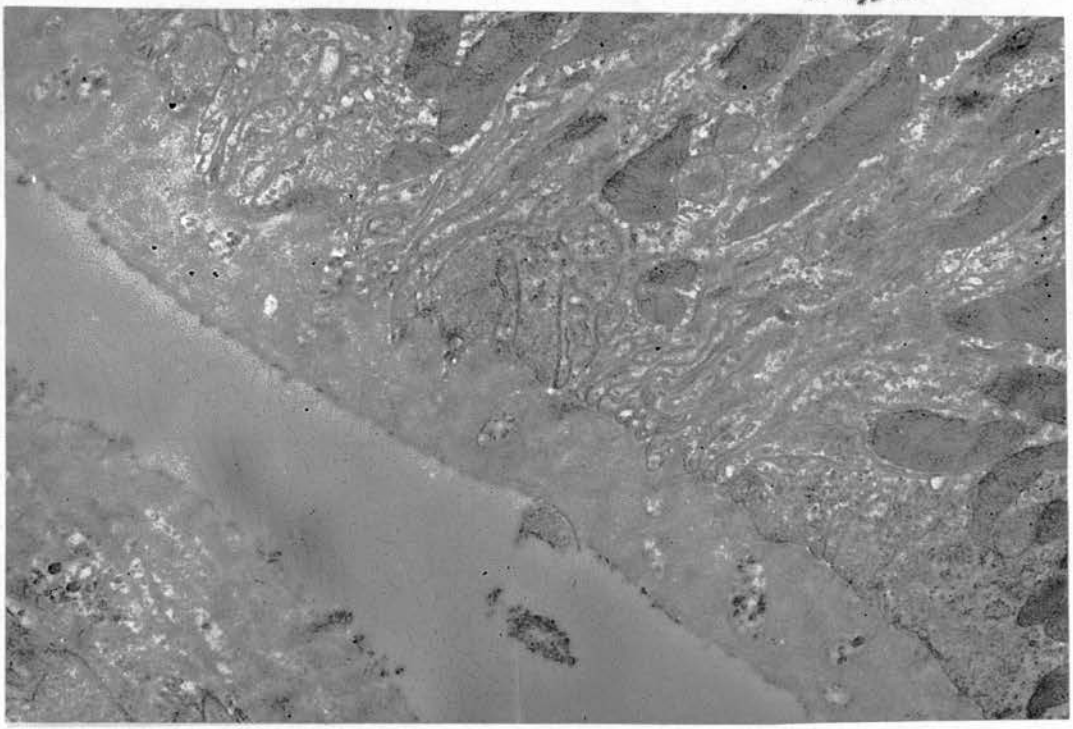


Fig. 164. Hydration experiment: Rat 6. Pars recta of a proximal tubule. The basement membrane is very markedly thickened and the basal cell foldings appear embedded in it.
x 15,000

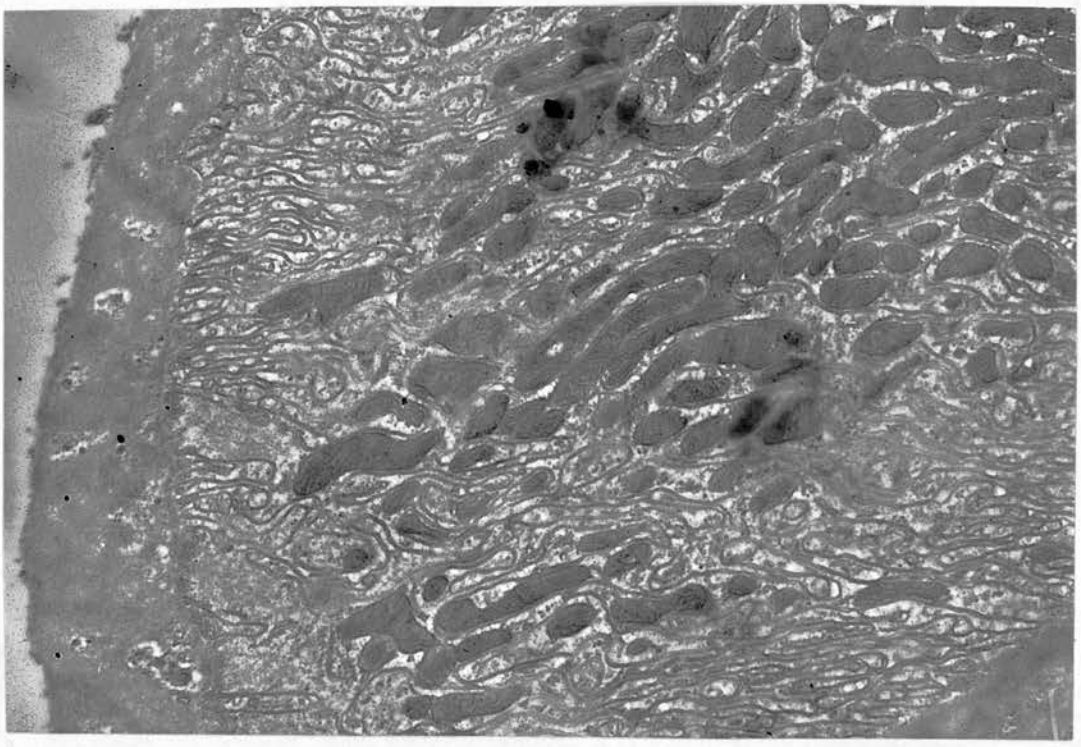


Fig. 165. Hydration experiment: Rat 4. Basement membrane of pars recta of a proximal tubule. x 10,000

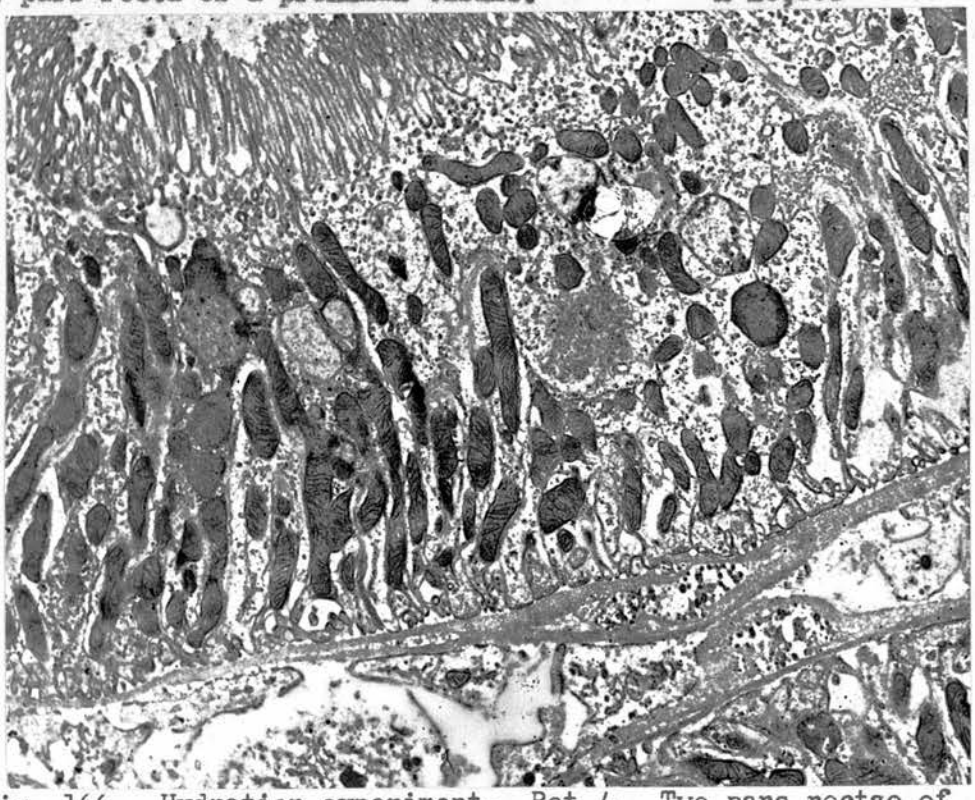


Fig. 166. Hydration experiment. Rat 4. Two pars rectae of proximal tubule. The basement membrane in each one is thickened and looks fibrillary and is split for a short distance, creating a space that contains numerous osmiophilic granules. x 6,000

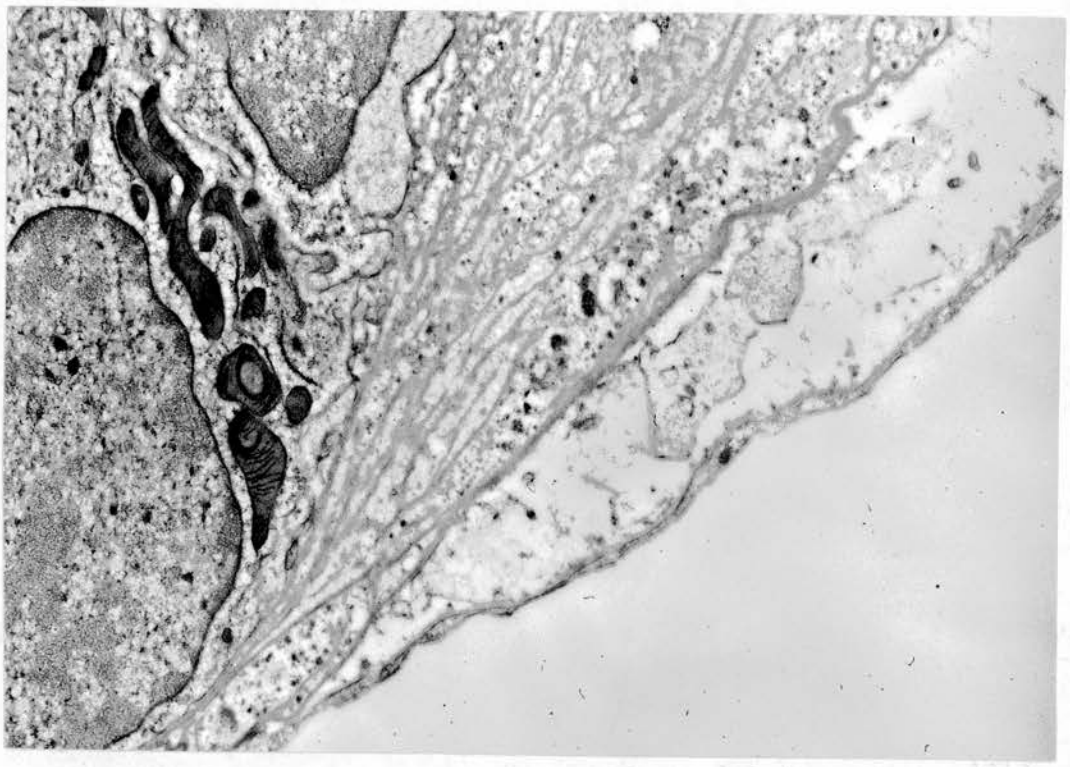


Fig. 167. Hydration experiment: Rat 4. Pars recta of a proximal tubule. Note that the basement membrane is split up and is continued as a sheaf of fibres with structureless densely osmiophilic granules in between. x 9,000

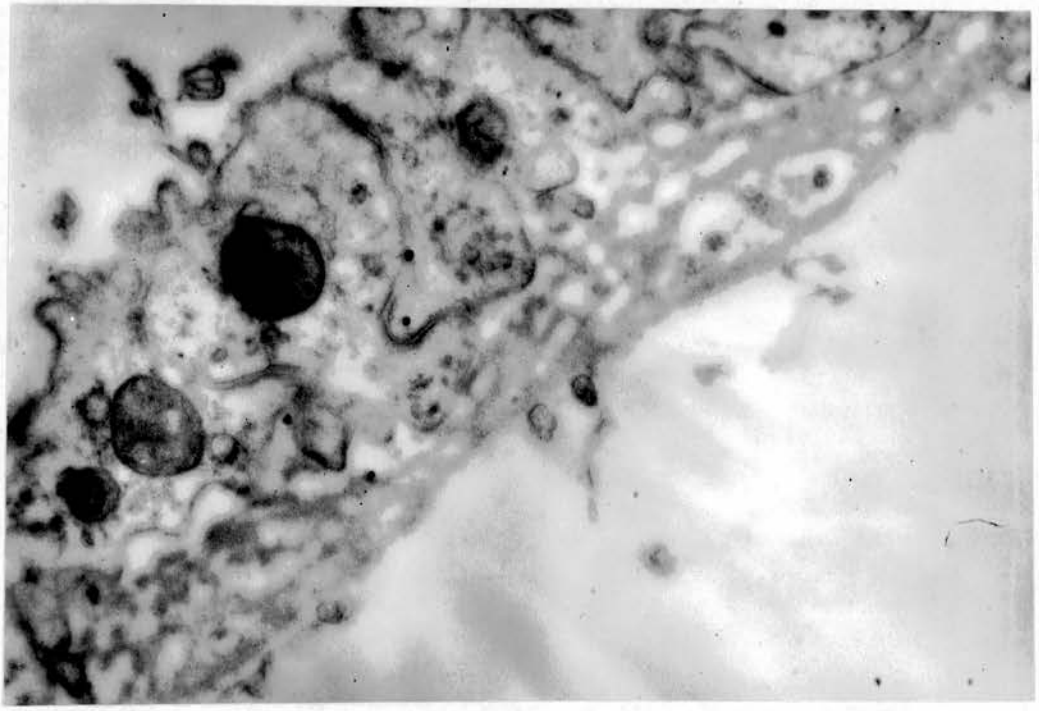


Fig. 168. Hydration experiment: Rat 3. Thin segment of the loop of Henle in the outer medulla. The basement membrane is thick and fibrillar and looks like a meshwork. x 30,000

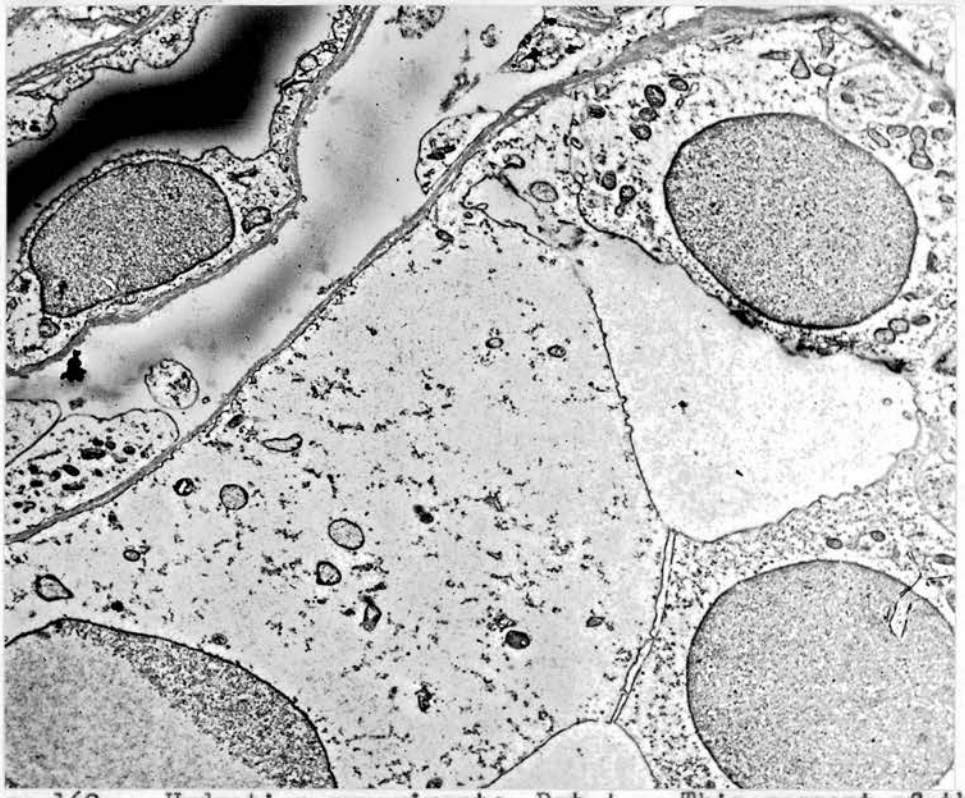


Fig. 169. Hydration experiment: Rat 4. Thin segment of the loop of Henle in the outer medullary zone. The cell on the left is very swollen and its cytoplasm rarified. The basement membrane of the tubule is thickened, but not particularly so beneath the swollen cell. x 2,500

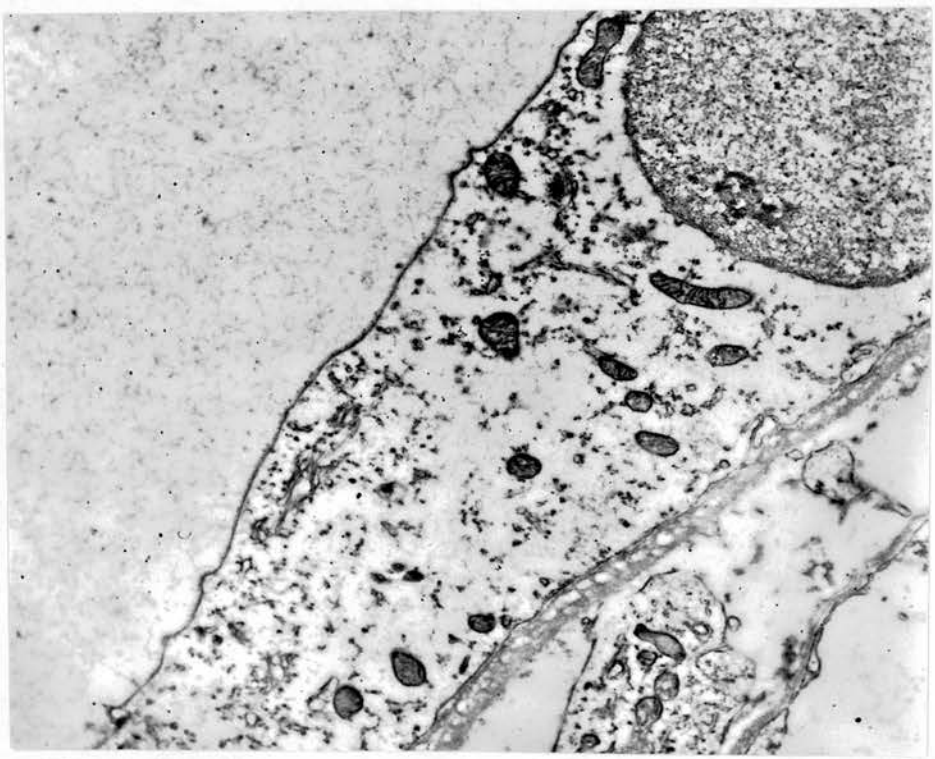


Fig. 170. Hydration experiment: Rat 4. Thin segment of the loop of Henle in the outer medulla. Note that the basement membrane is thickened and that the basal cell foldings are flattened out. x 9,000

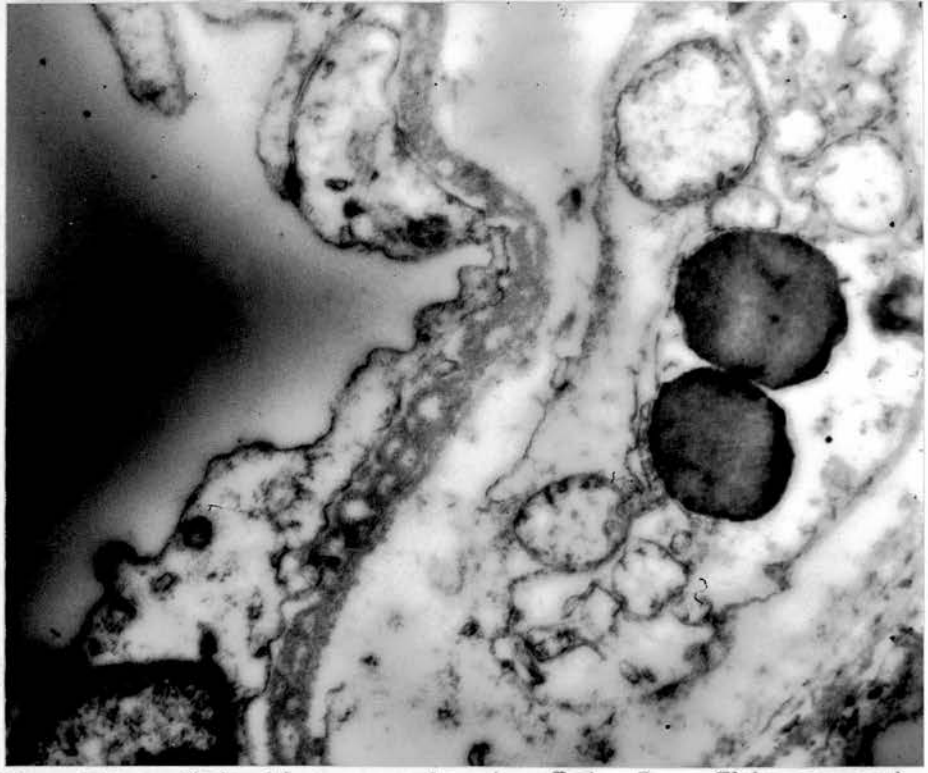


Fig. 171. Hydration experiment. Rat. 5. Thin segment of a loop of Henle in the inner medulla. Note the thick, fibrillar basement membrane. x 24,000

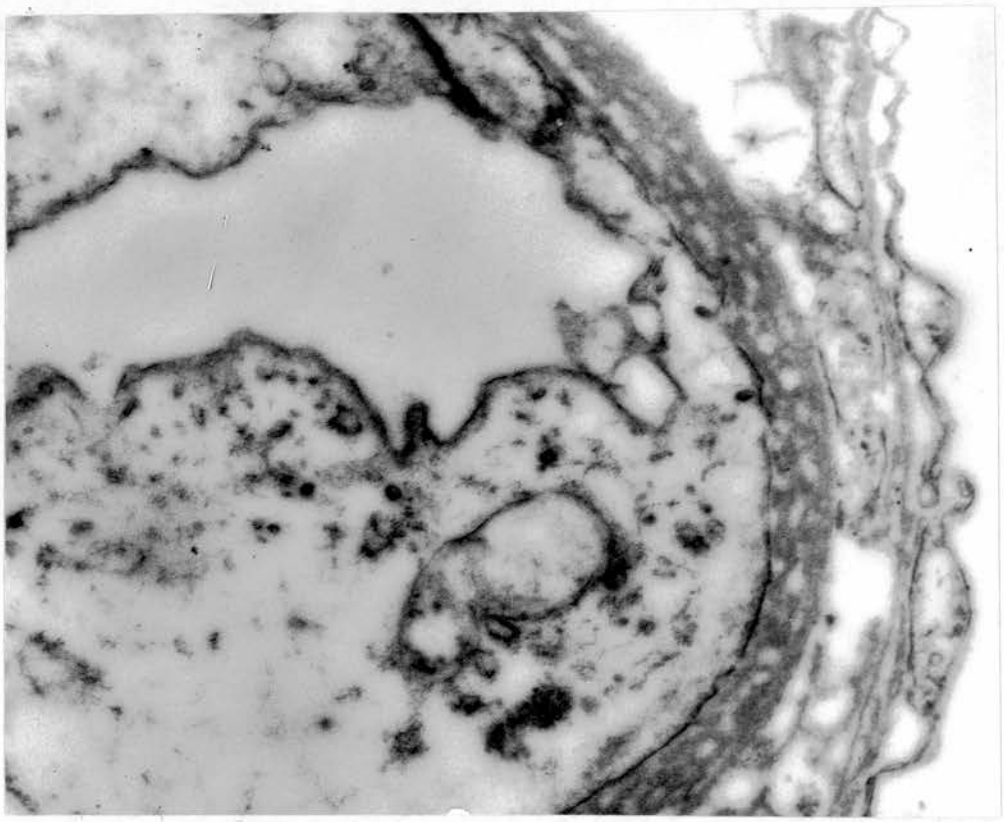


Fig. 172. Hydration experiment: Rat 5. Thin segment of a loop of Henle and a capillary in the inner medulla. Note the thick basement membrane of the thin segment, which looks like a meshwork, and the absence of the basal cell foldings. x 24,000

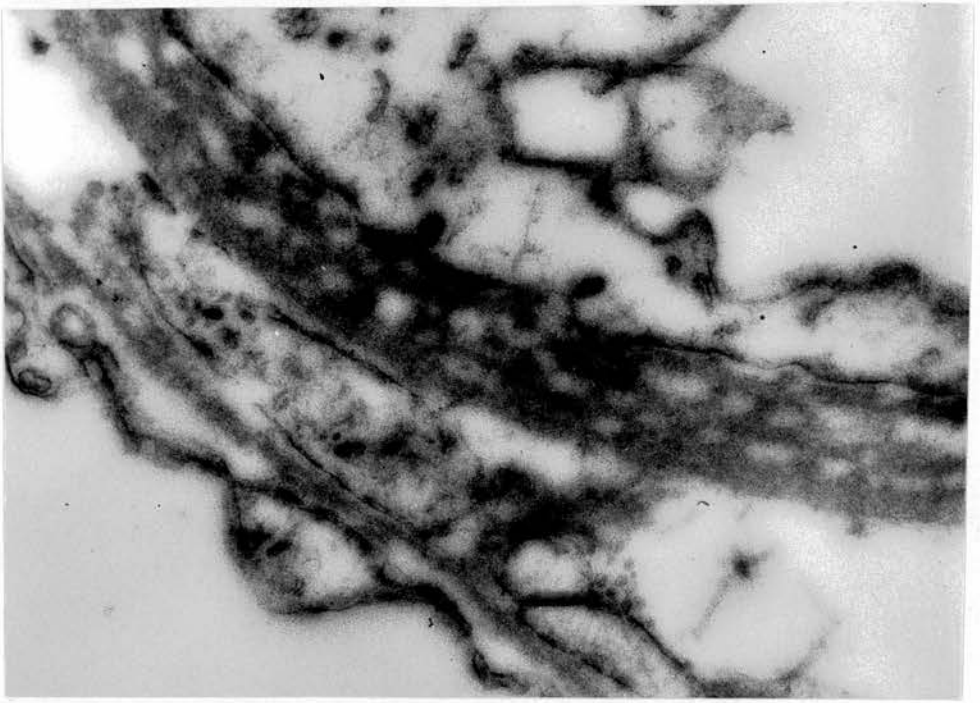


Fig. 173. Hydration experiment: Rat 5. Markedly thickened basement membrane of a thin segment of a loop of Henle in the inner medulla. The adjacent efferent capillary looks normal. x 45,000

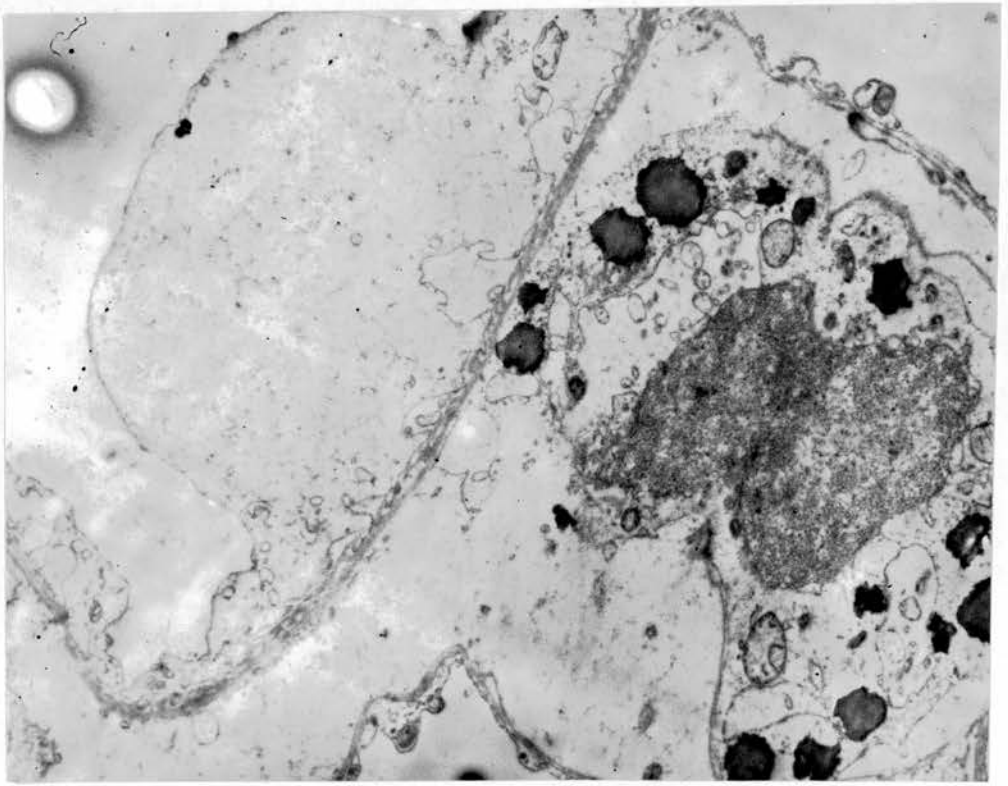


Fig. 174. Hydration experiment: Rat 5. Swollen cell in a thin segment of a loop of Henle in the inner medulla. An interstitial cell is seen on the right. x 12,000

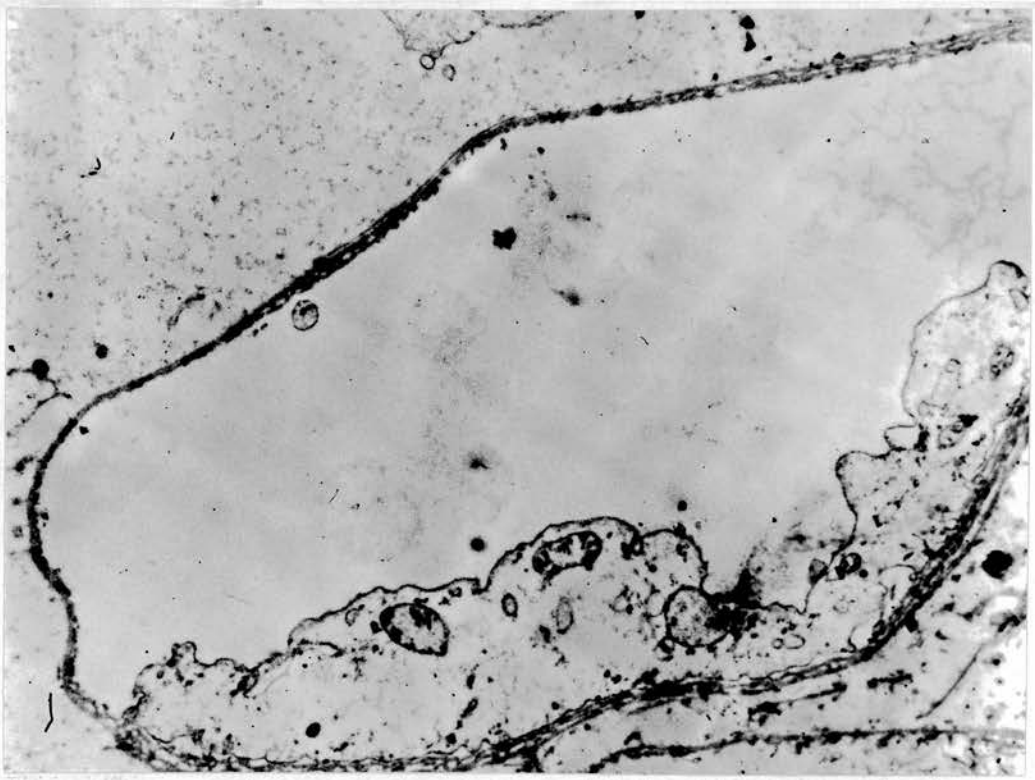


Fig. 175. Hydration experiment: Rat 5. Thin segment of a loop of Henle in the inner medulla. The basement membrane is moderately thickened and fibrillar. A large part of the basement membrane is bare, having lost its epithelial lining. x 9,000

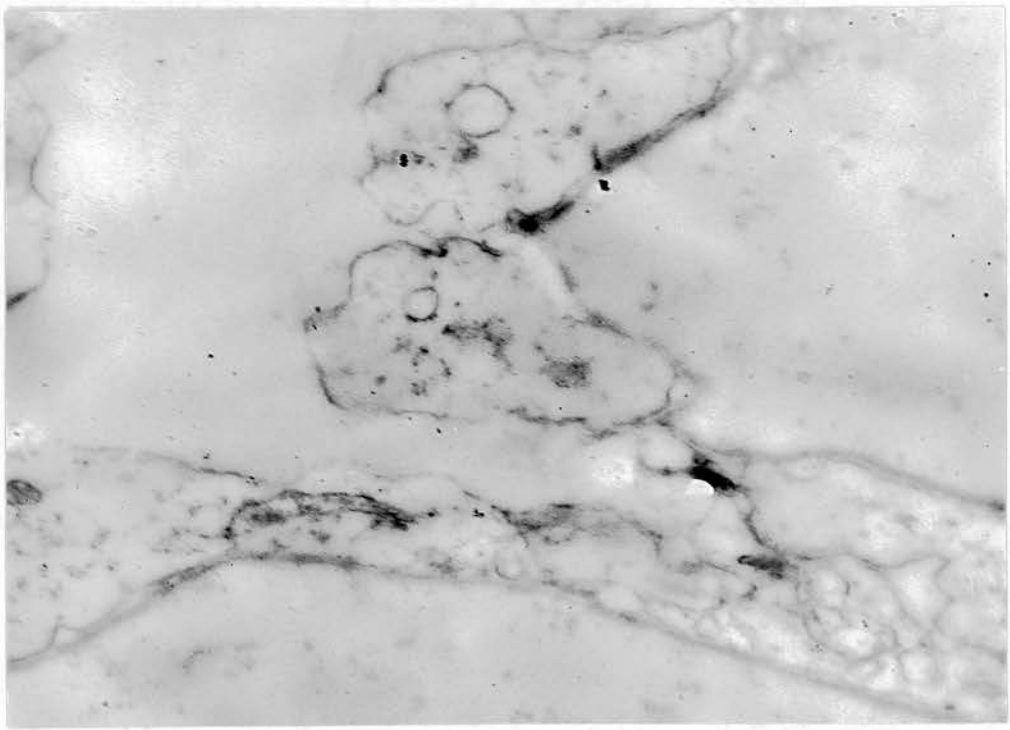


Fig. 176. Hydration experiment: Rat 3. Thin segment of a loop of Henle in the inner medulla. The basement membrane is normally thin and appears as a single line. Compare with Fig. 172, which is of the same magnification. x 24,000

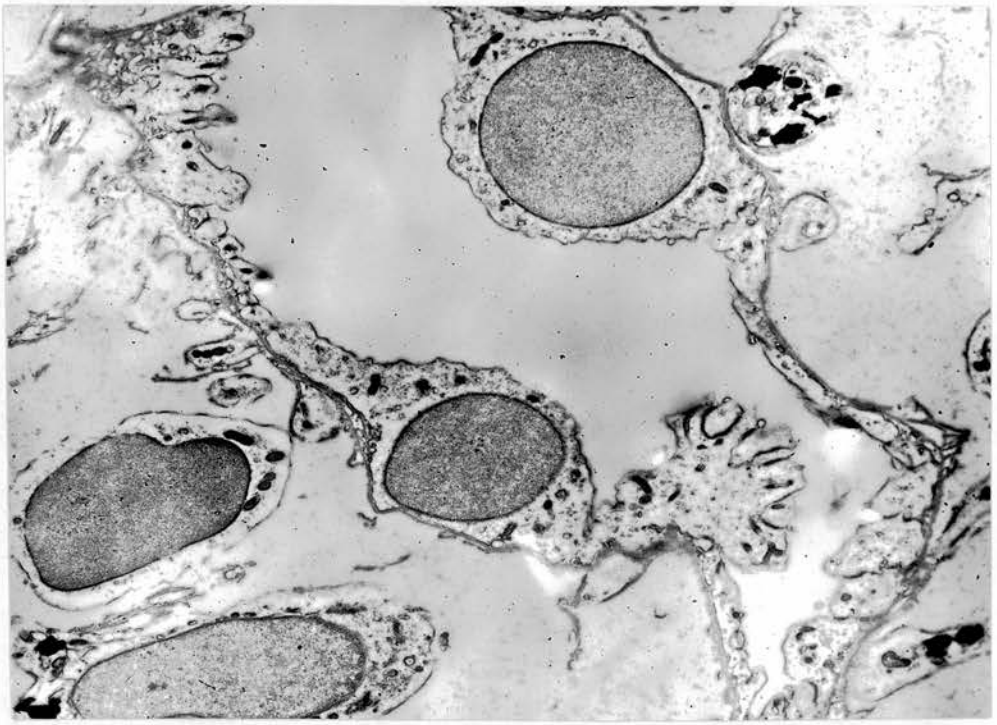


Fig. 177. Hydration experiment: Rat 3. Thin segment of a loop of Henle in the inner medulla. The basement membrane is not thickened, the basal cell foldings are flattened out; and a papilliform process is seen to project into the lumen x 2,500

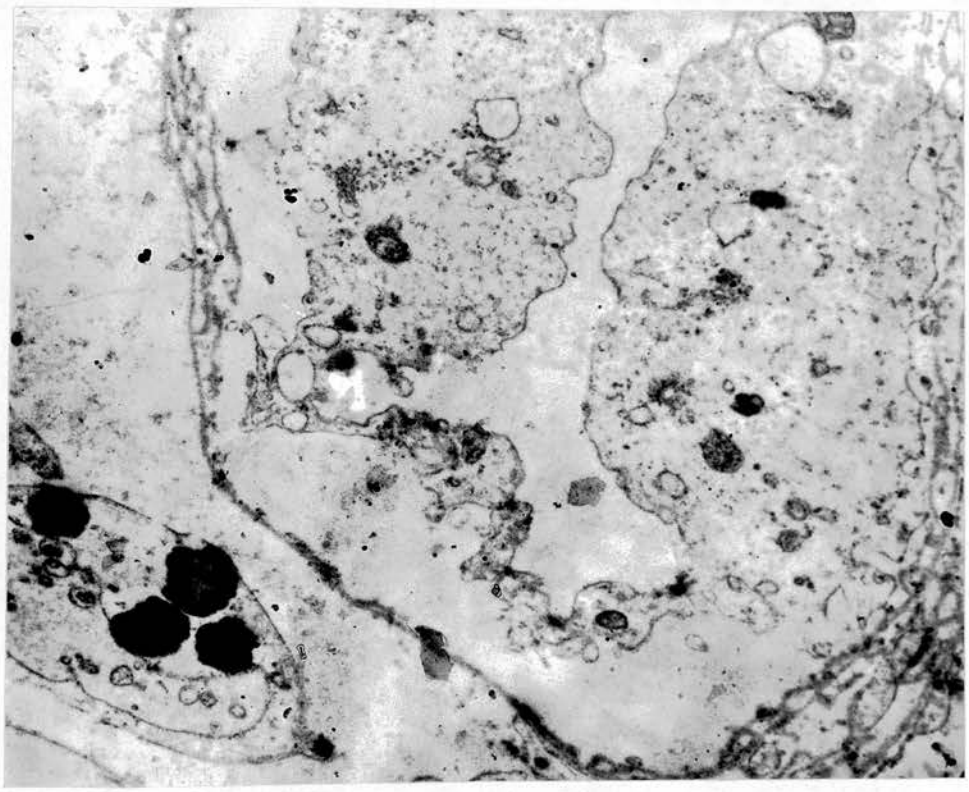


Fig. 178. Hydration experiment: Rat 5. Thin segment of a loop of Henle in the inner medulla. The basement membrane is not thickened; the cells are separated from the basement membrane and their cytoplasm contains a number of vacuoles mostly related to mitochondria. x 12,000

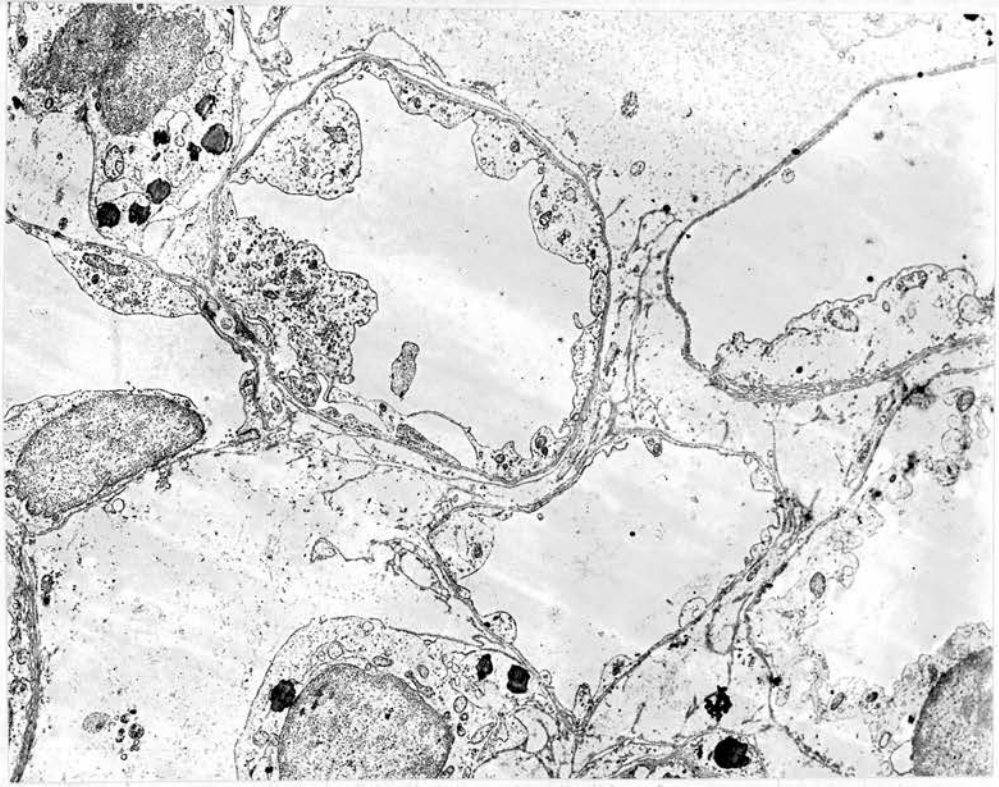


Fig. 179. Hydration experiment. Rat 5. A low power electron micrograph showing a thick capillary in the centre and a thin capillary below it. On the right, two thin segments can be seen; in the low-power one, the epithelium is separating from the normal basement membrane, while in the upper one, an extensive area of the basement membrane is bare, because its lining cell has been shed off.
x 4,000

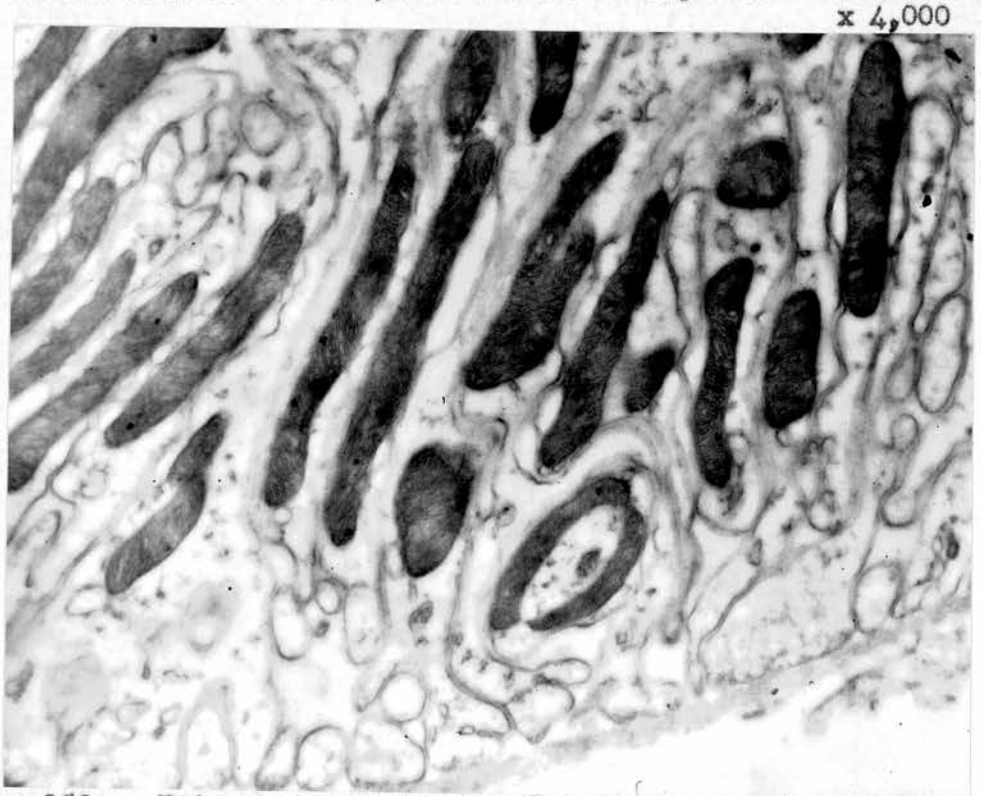


Fig. 180. Hydration experiment: Rat 4. The basal part of a cell of the thick segment of a loop of Henle. Note the normal basement membrane.
x 24,000

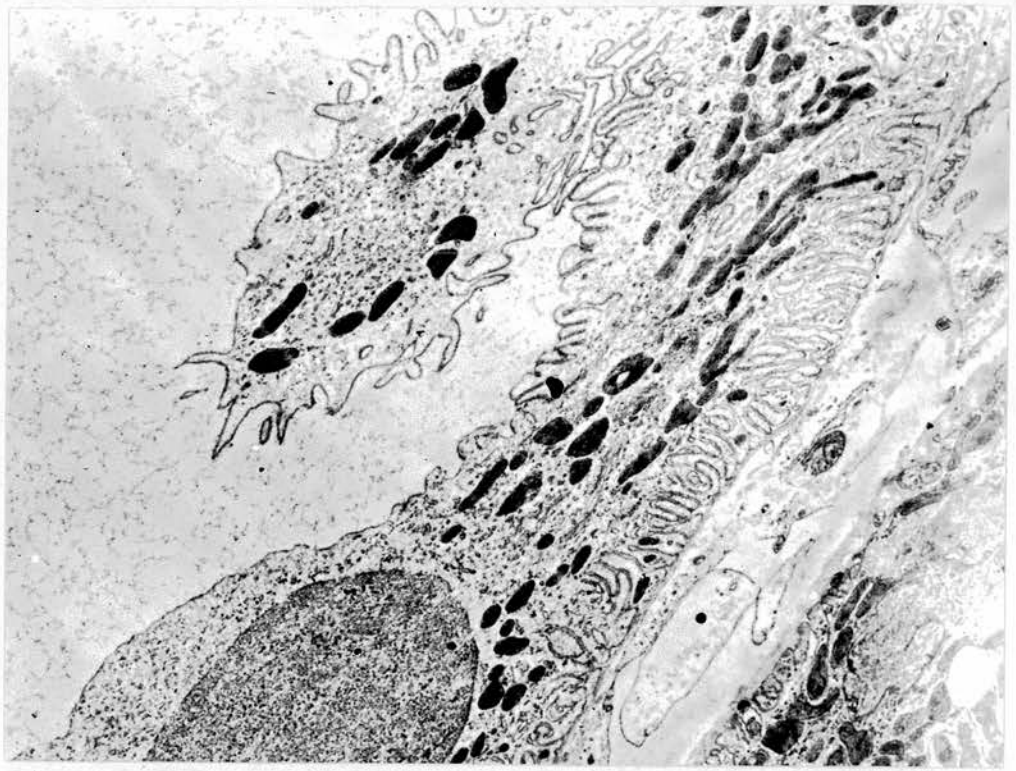


Fig. 181. Hydration experiment: Rat 1. A dark cell in an outer medullary collecting tubule. A papilliform process projects into the lumen and the luminal and basal cell surfaces are thrown into a large number of folds. x 9,000

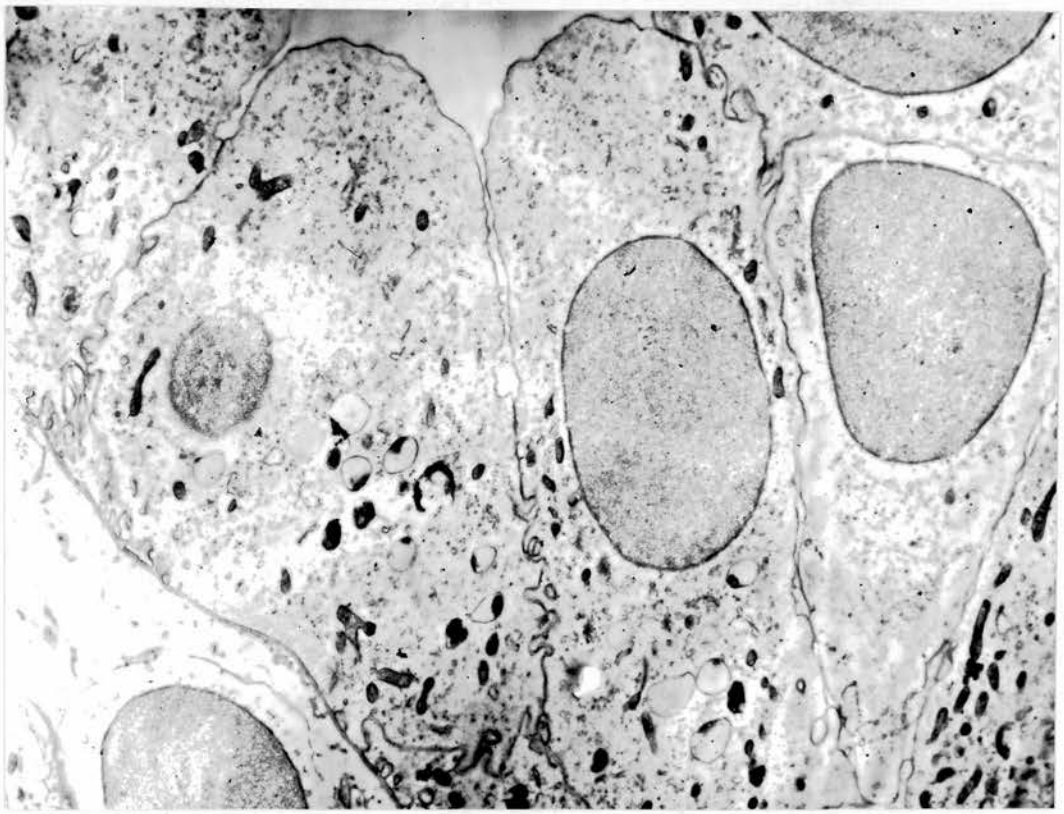


Fig. 182. Hydration experiment: Rat 3. Light cells from an inner medullary collecting tubule. Many vacuoles with clear contents are seen in the cytoplasm. Some of these vacuoles have a very dark, dense body at their periphery. x 6,000

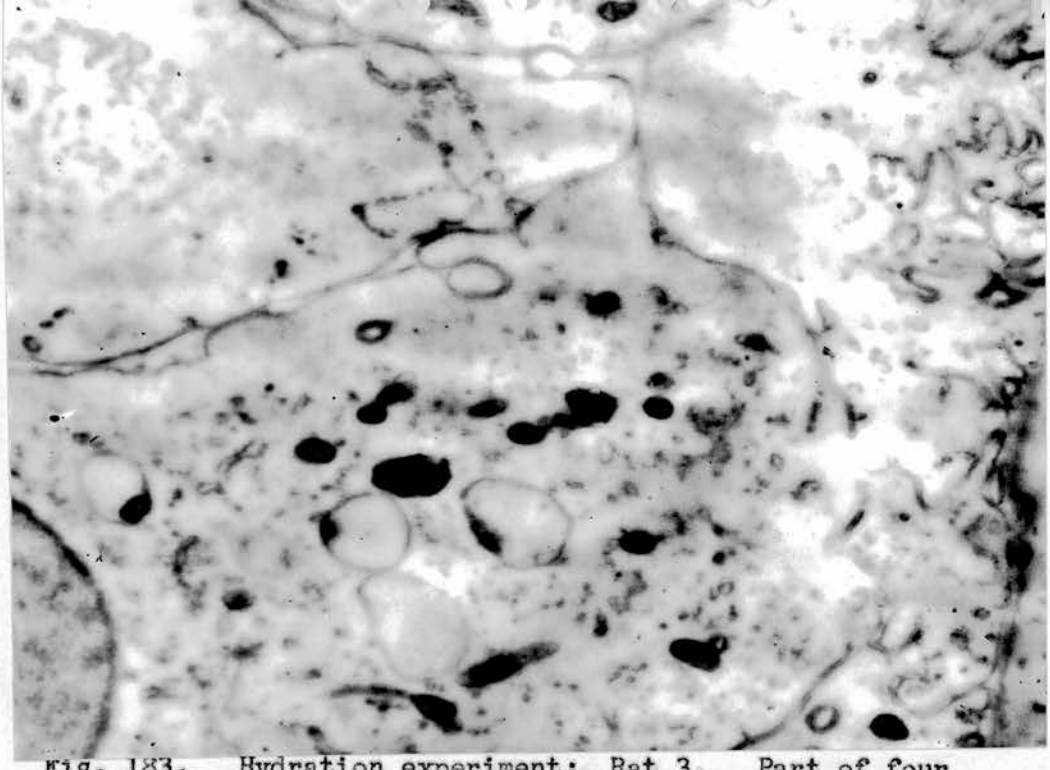


Fig. 183. Hydration experiment: Rat 3. Part of four adjacent light cells of an inner medullary collecting tubule. Note the dense cytoplasmic granules, the granulated and the non-granulated vacuoles and the vacuoles that lie between the plasma membranes of adjacent cells. 15,000

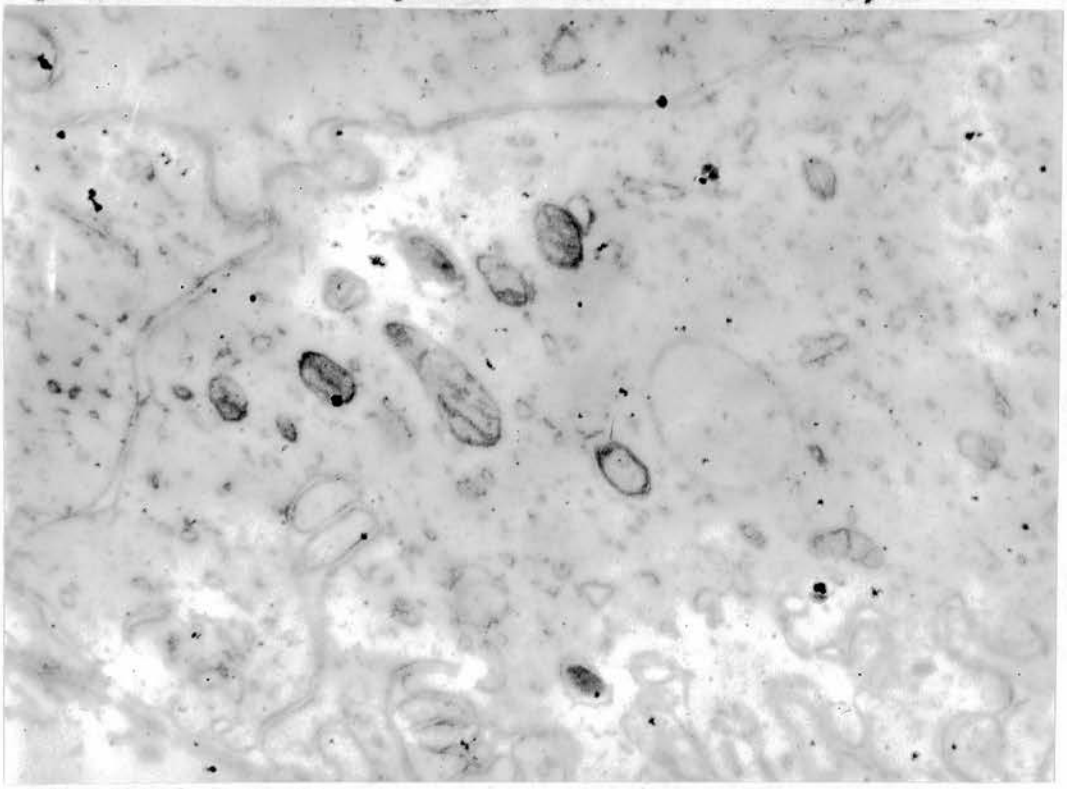


Fig. 184. Hydration experiment: Rat 3. Part of three adjacent light cells of an inner medullary collecting tubule. Two vacuoles are clearly seen in the labyrinthine space, beneath the basal cell membrane. The cytoplasm contains many vacuoles; in one of them a dense central body is seen and in another, a dense peripheral rim is observed. x 30,000

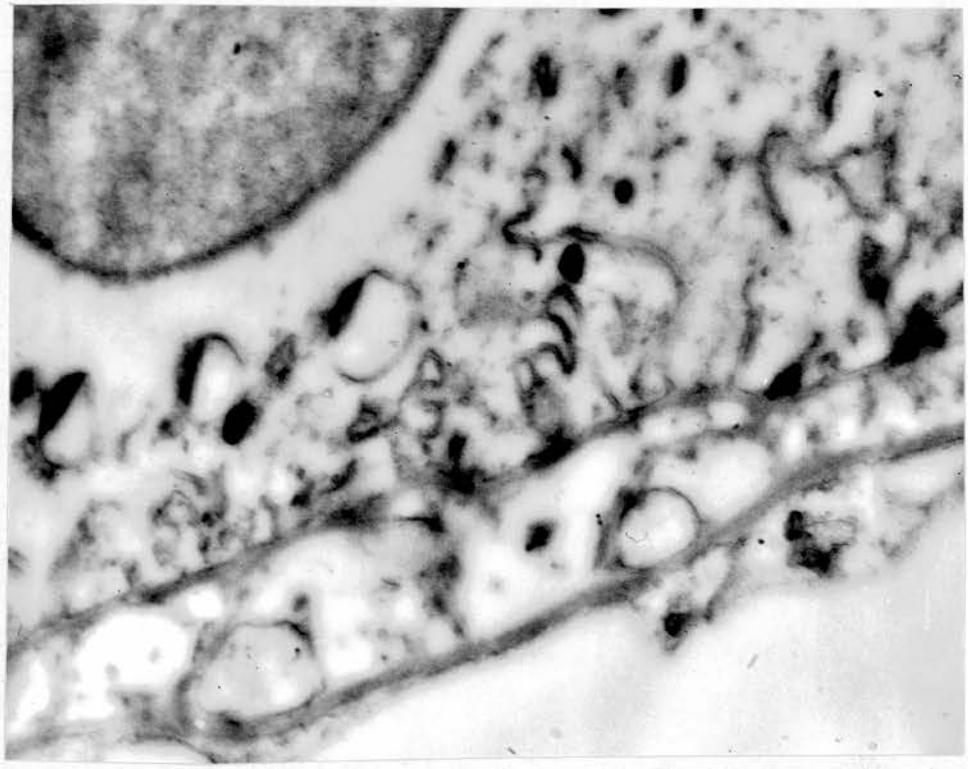


Fig. 185. Hydration experiment. Rat 3. The basal part of a light cell of an inner medullary collecting tubule. Note the vacuoles in the cellular cytoplasm and in the interstitial space in contact with the capillary basement membrane. Note the crescentic, dense body at the periphery of some of the vacuoles. x 30,000

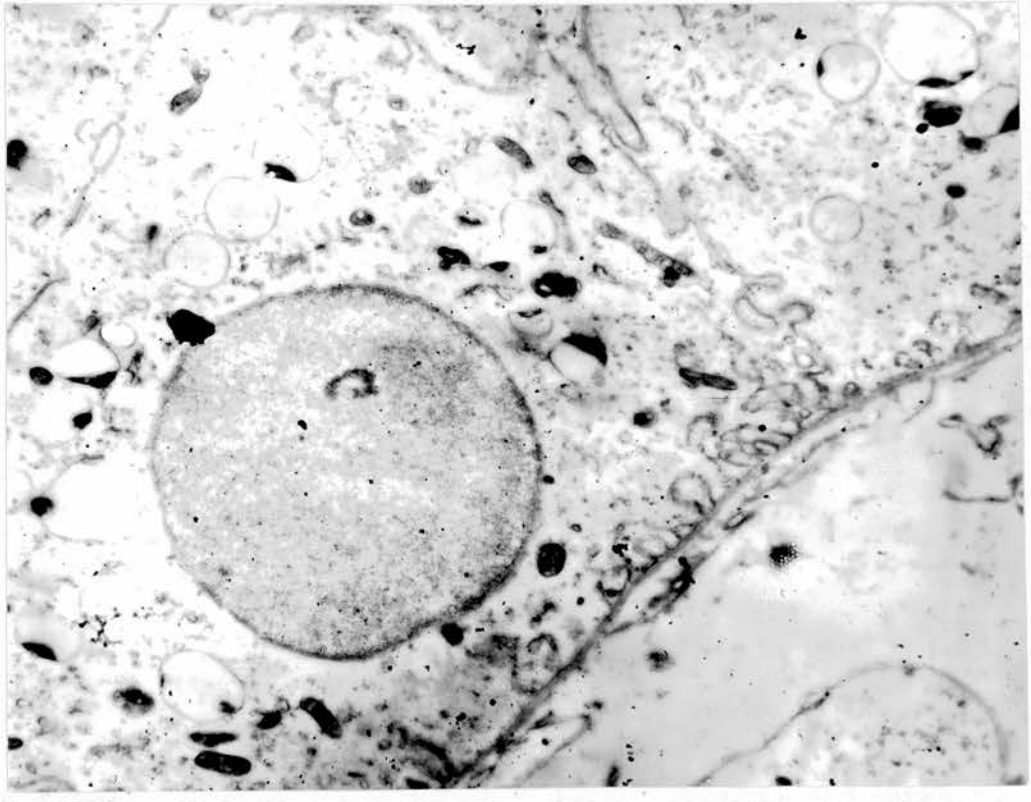


Fig. 186. Hydration experiment. Rat 3. Light cells of an inner medullary collecting tubule. The cytoplasm contains numerous vacuoles that have a densely osmiophilic crexcentic body at the periphery. x 9,000

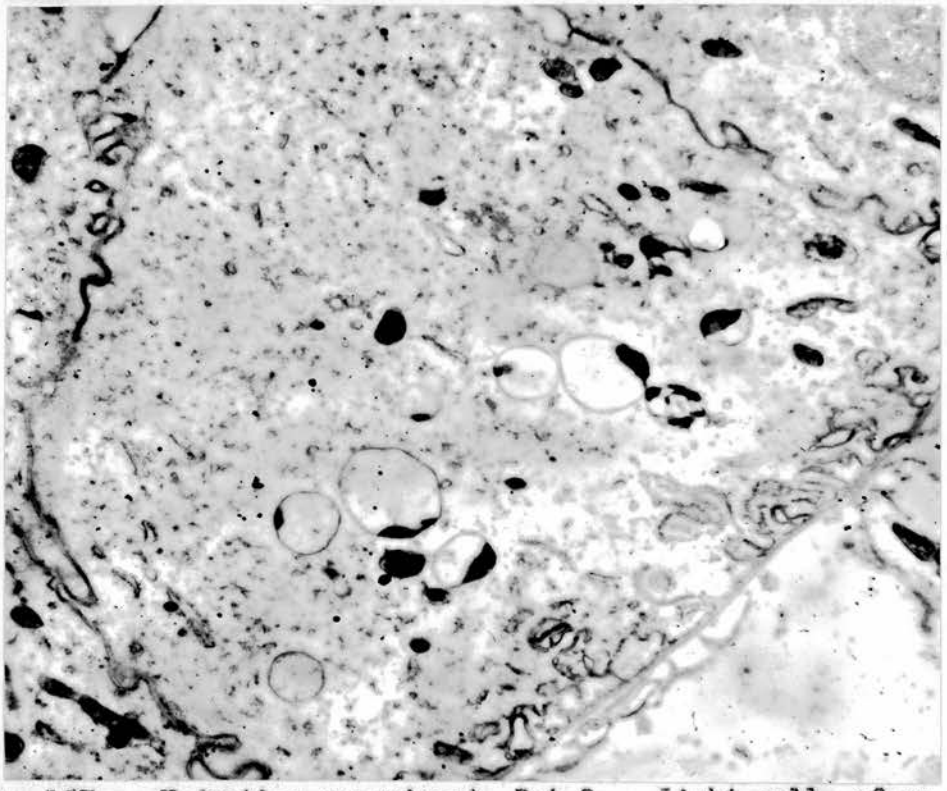


Fig. 187. Hydration experiment. Rat 3. Light cell of an inner medullary collecting tubule. Numerous cytoplasmic vacuoles are seen: the majority have a densely osmiophilic peripheral body. x 9,000

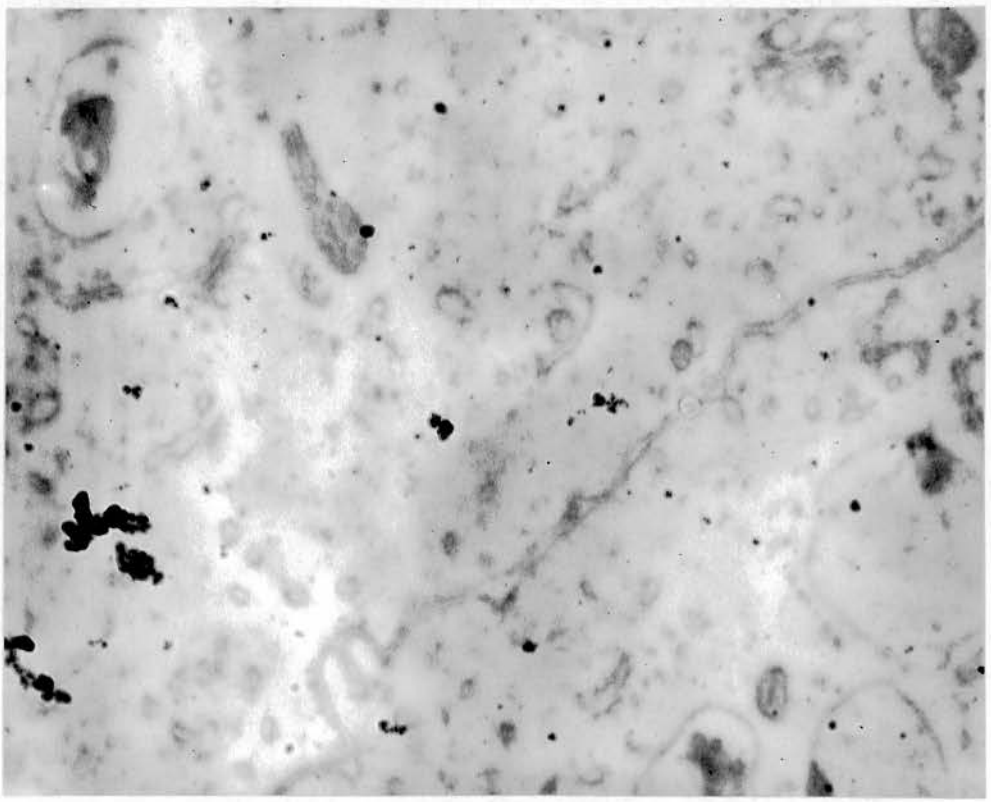


Fig. 188. Hydration experiment. Rat 3. Part of two adjacent light cells of an inner medullary collecting tubule, some cytoplasmic vacuoles have peripheral dense granules while the vacuole on the left side has a central granule. x 27,000

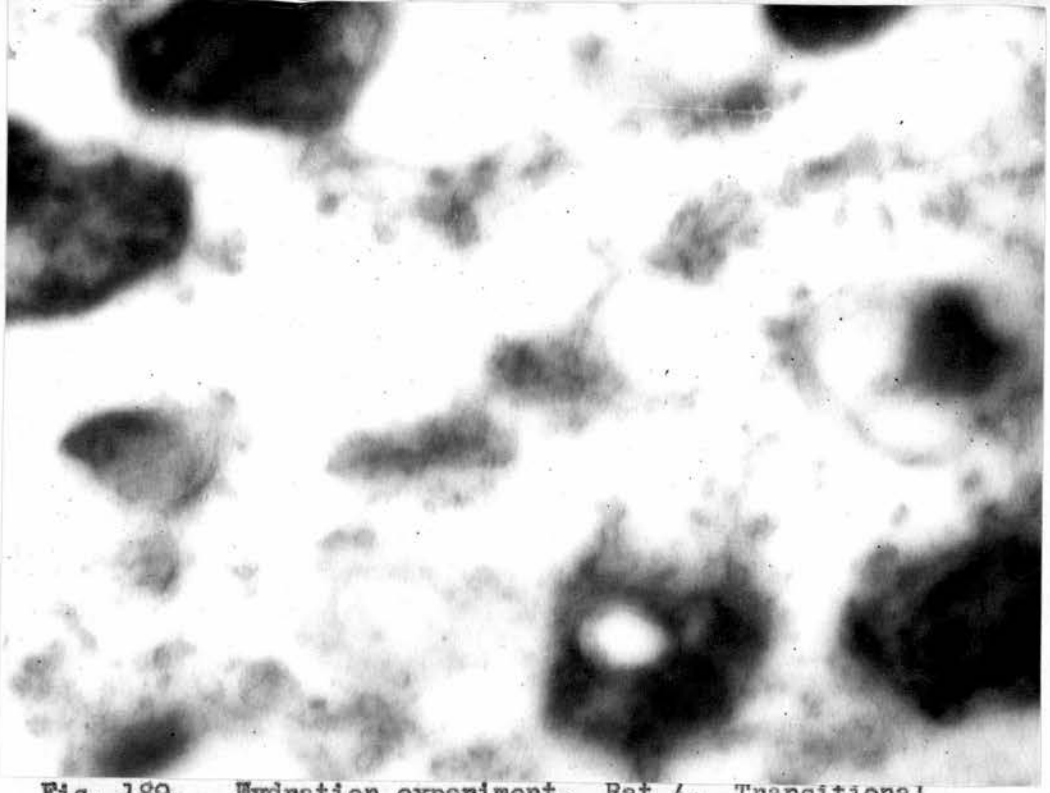


Fig. 189. Hydration experiment. Rat 4. Transitional stages from a mitochondrion into which a dense body is present at one side, to a vacuolated granule, to a vacuole with a small peripheral granule can be seen in this light cell from an inner medullary collecting tubule. x 40,000

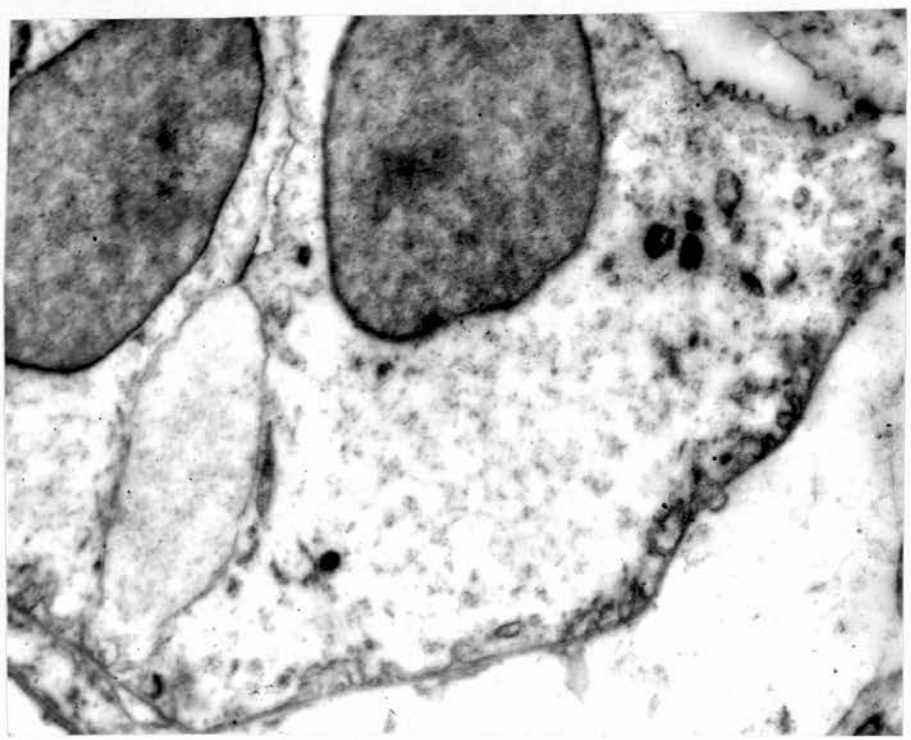


Fig. 190. Hydration experiment. Rat 5. An inner medullary collecting tubule showing partial lateral separation between adjacent cells, complete on the basement membrane side, but the cells are still adherent on the luminal side at the terminal bar. x 9,000

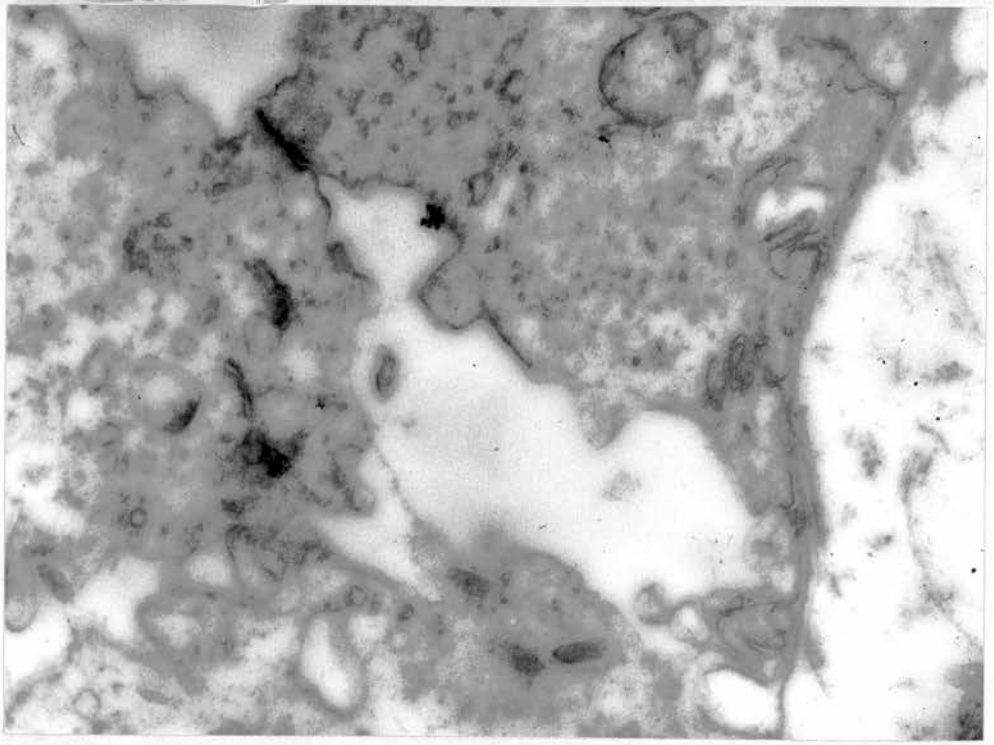


Fig. 191. Hydration experiment. Rat 5. Two adjacent cells in an inner medullary collecting tubule showing lateral separation, but they are still adherent at the terminal bar. x 24,000

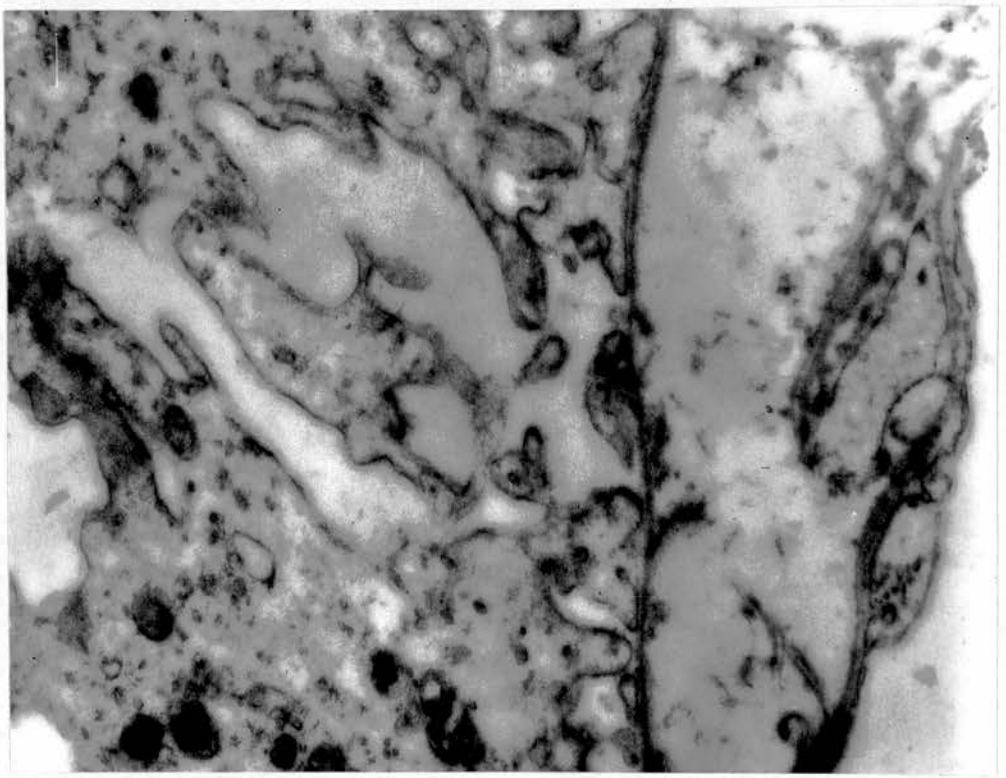


Fig. 192. Hydration experiment. Rat 5. Although these two cells in an inner medullary collecting tubule are widely separated, they are still adherent to each other on the luminal side at the terminal bar. x 24,000

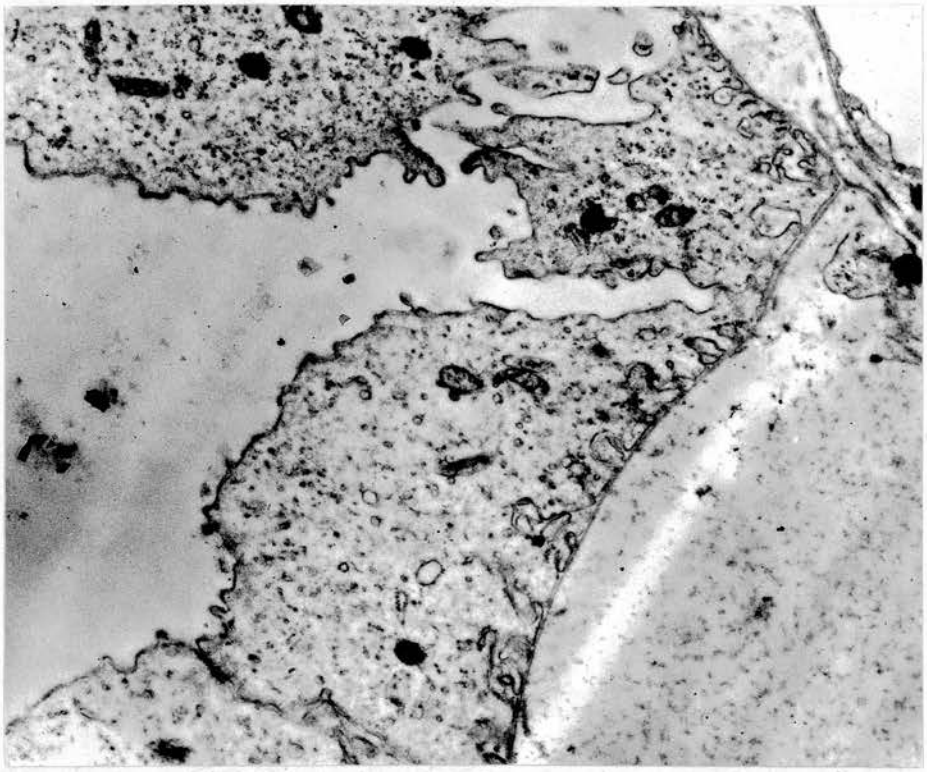


Fig. 193. Hydration experiment. Rat 5. Part of an inner medullary collecting tubule showing complete lateral separation between adjacent cells. x 9,000

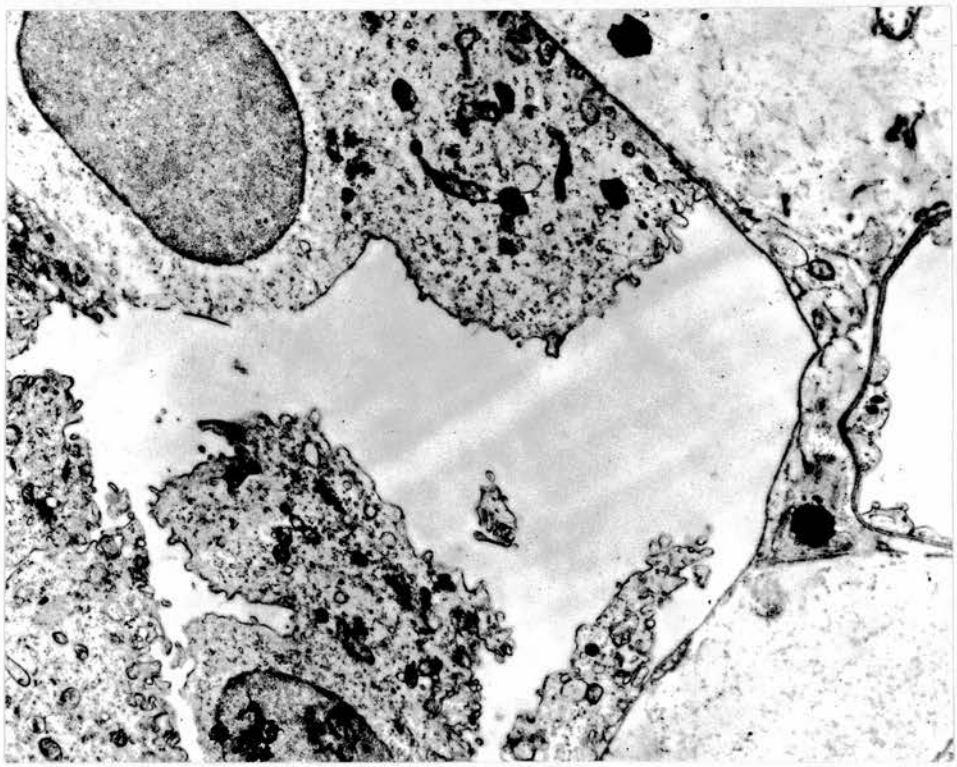


Fig. 194. Hydration experiment. Rat 5. Part of an inner medullary collecting tubule showing a bare part of the basement membrane. x 6,000

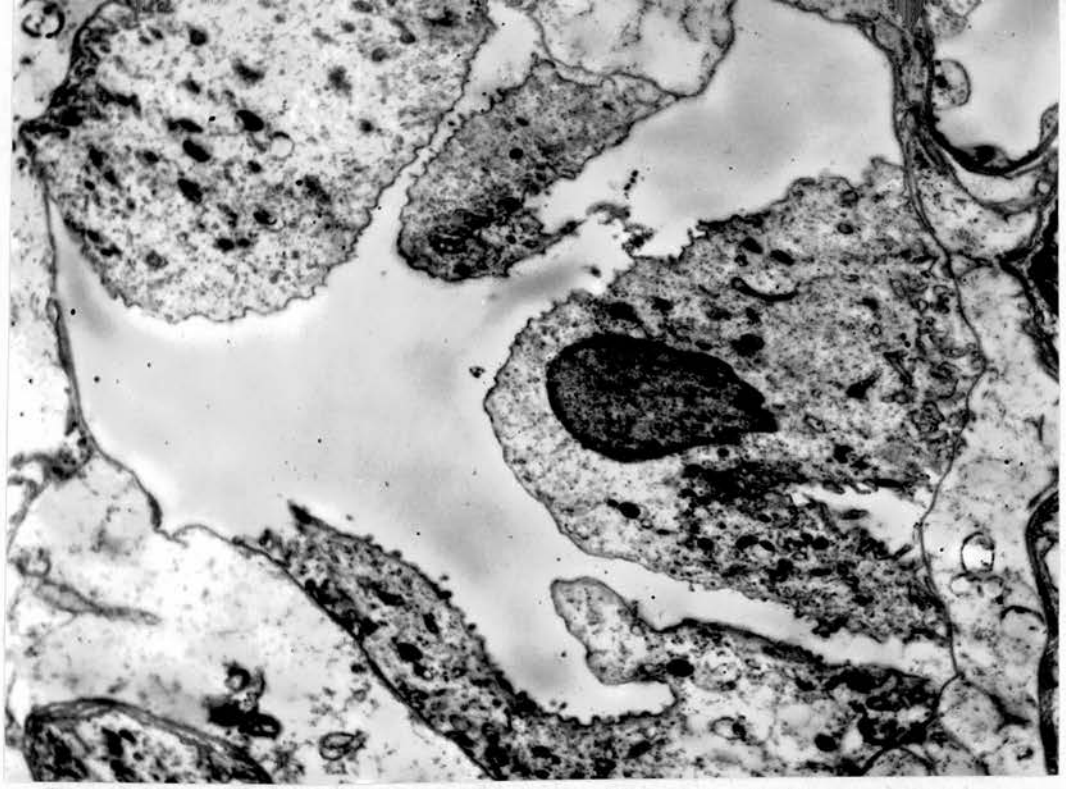


Fig. 195. Hydration experiment. Rat 5. An inner medullary collecting tubule showing complete separation of its lining cells from each other and resulting in the appearance of four bare areas of the basement membrane. The cell on the right clearly shows the thick primary process and the fine secondary processes. Numerous granulated vacuoles are seen in the cells and in the interstitial space. x 4,000

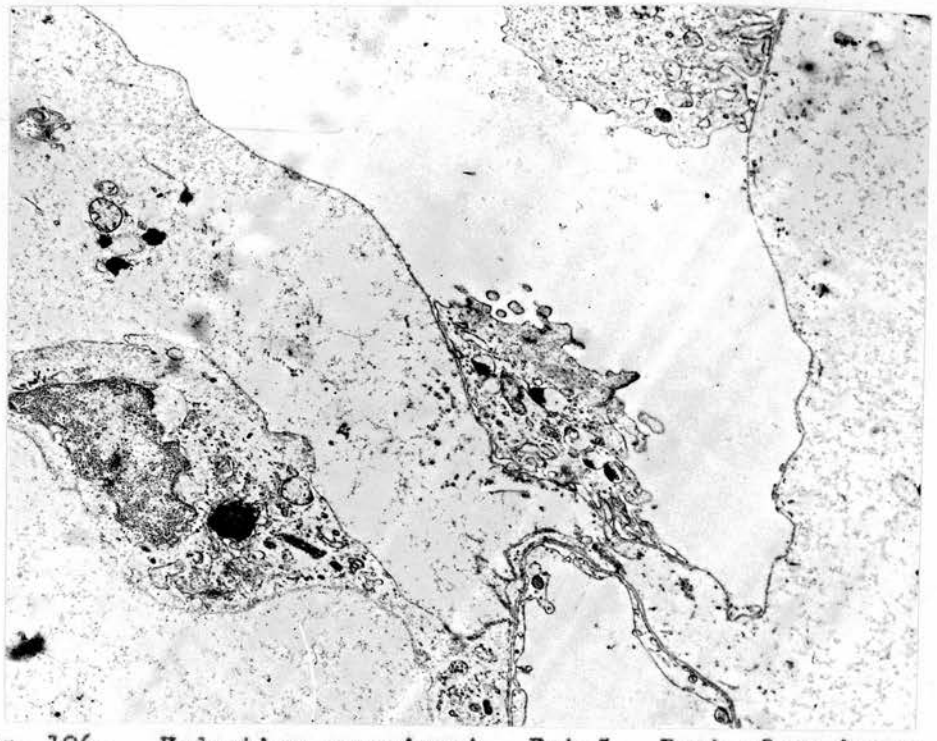


Fig. 196. Hydration experiment. Rat 5. Part of an inner medullary collecting tubule showing extensive stretches of the basement membrane completely bare. Note the granulated vacuoles in the cellular cytoplasm. x 6,000

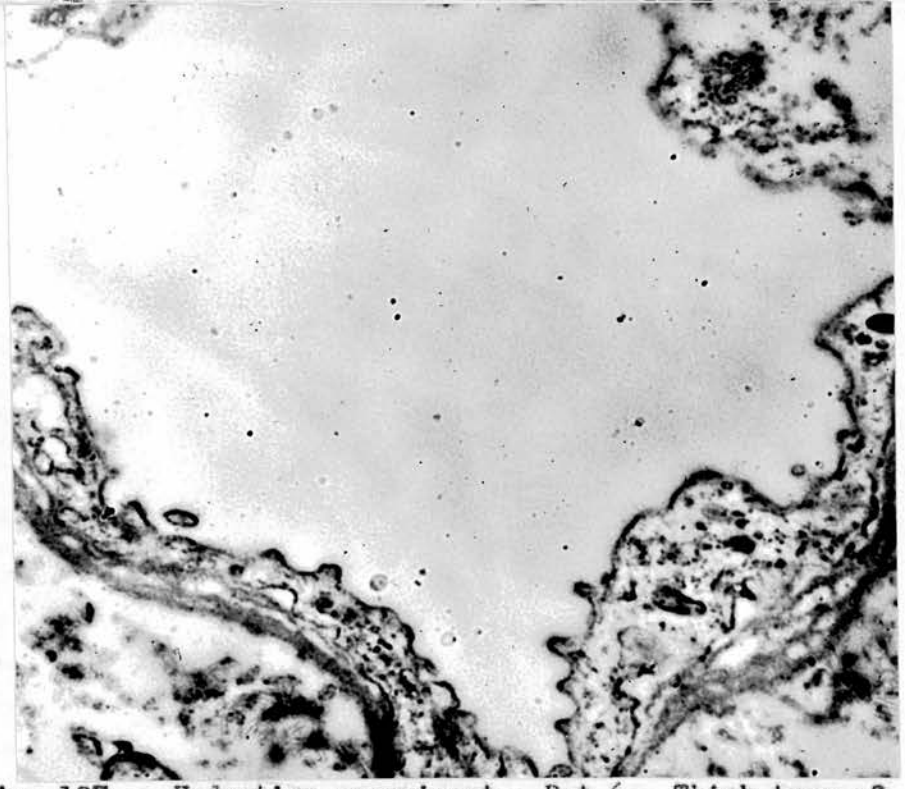


Fig. 197. Hydration experiment. Rat 6. Thick type of medullary capillary. The basement membrane is about twice the normal thickness. x 15,000

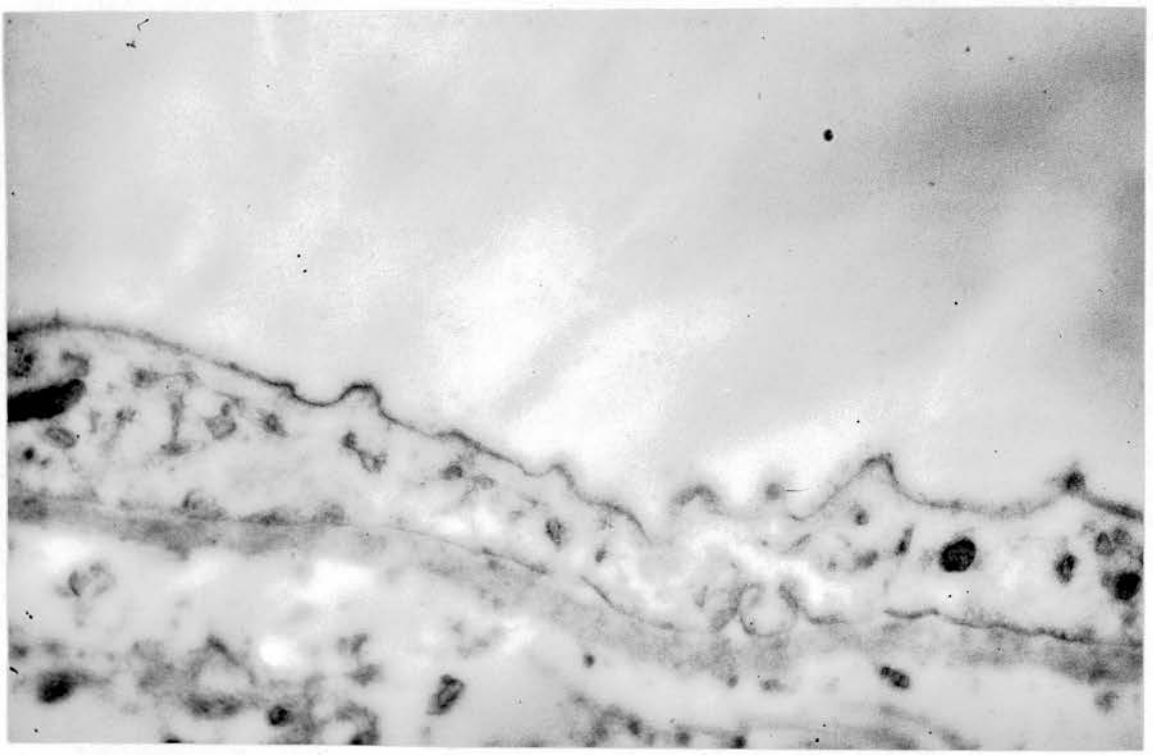


Fig. 198. Hydration experiment. Rat 3. Thick type of medullary capillary. The basement membrane is more than double the normal thickness. x 24,000

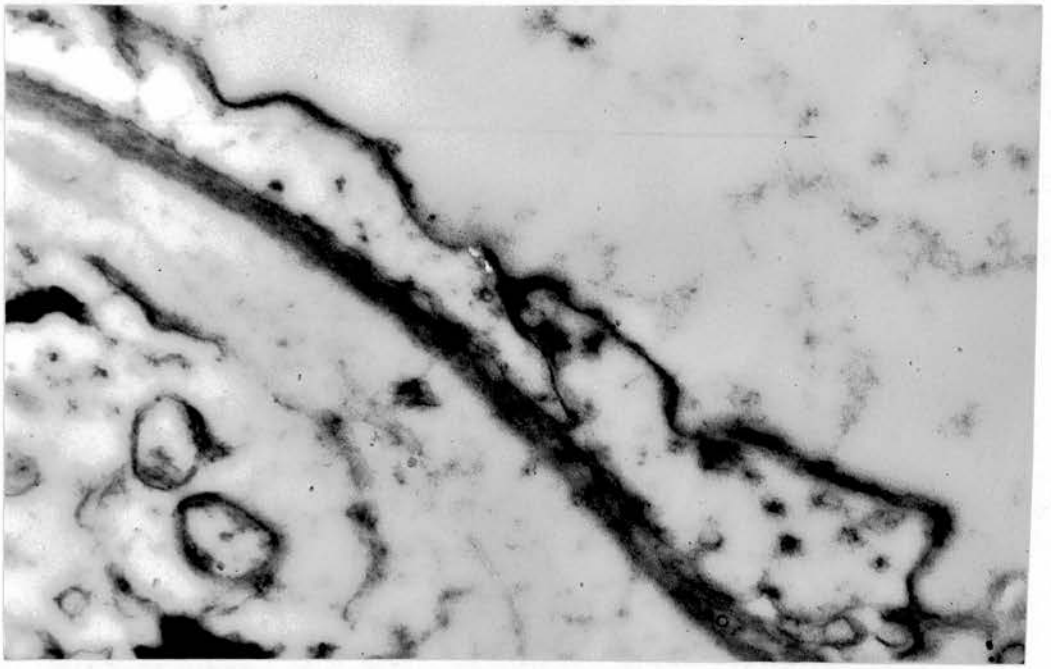


Fig. 199. Hydration experiment. Rat 6. Thick capillary in the inner stripe of the outer medulla. Note the thickness of the basement membrane. x 24,000

Fig. 200. Hydration experiment. Rat 5. Thin type of medullary capillary. The basement membrane is normally thin (Compare with Fig. 197,198, & 200). x 45,000

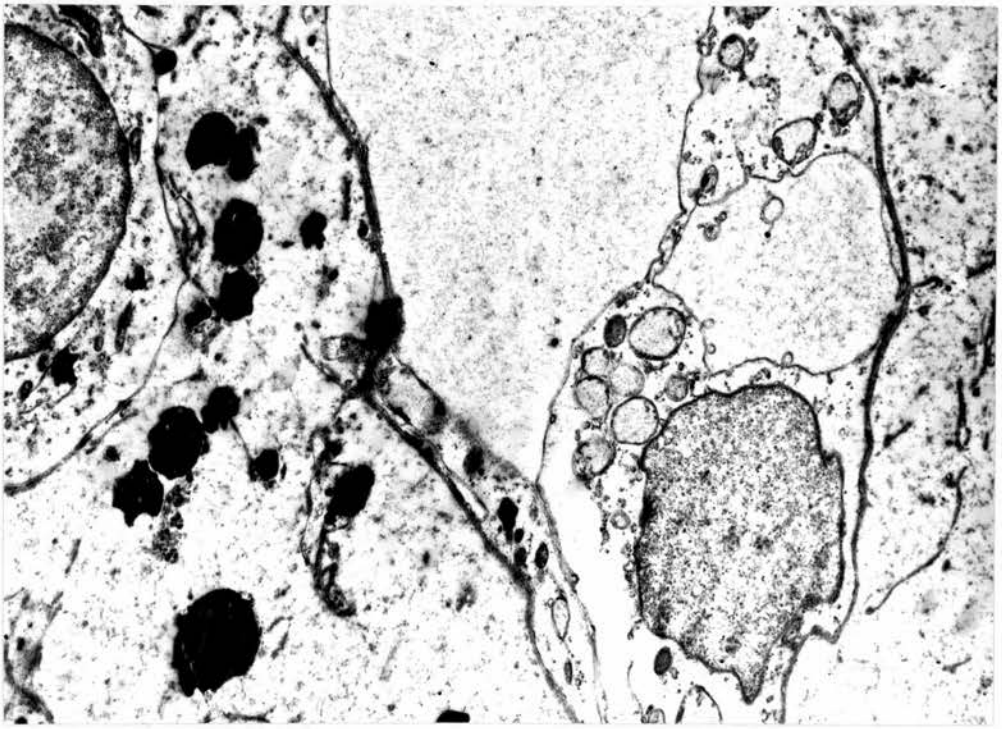


Fig. 201. Hydration experiment. Rat 4. A papillary capillary. The endothelium is swollen and contains a large vacuole on the right. x 6,000

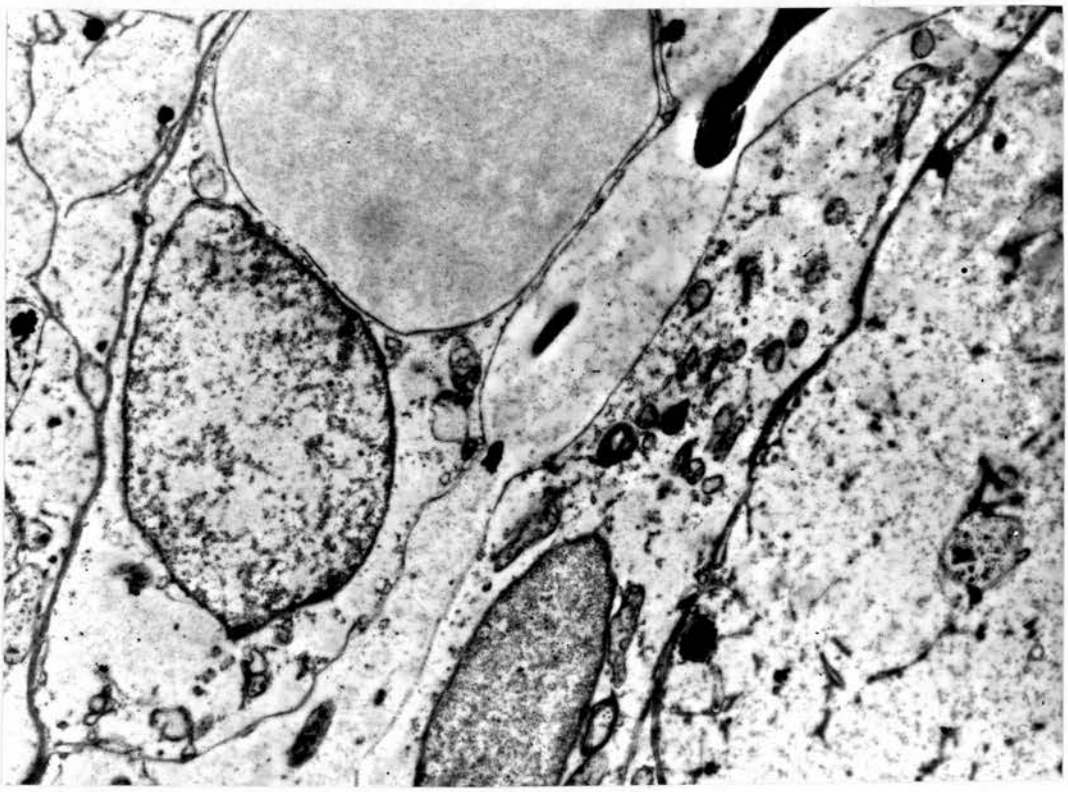


Fig. 202. Hydration experiment. Rat 4. A papillary capillary. The endothelium is swollen and contains a very large vacuole, larger than the nucleus. x 6,000

of vacuoles (Fig. 201). Some of these vacuoles were very large indeed, larger than the cell nucleus itself (Fig. 202).

II. Hydration followed by dehydration experiment:

The osmolality of the urine passed within the two hours following 48 hours dehydration is seen in Table (6).

Table 6.

Rat No.	Weight g.	Volume of water administered ml.	Duration of dehydration hours	Osmolality of urine passed in the last two hours m.Osm/Kg.
7	230	5	50	2540
8	250	20	50	2500
9	250	20	50	2600

On light microscopy: No abnormality detected.

On Electron microscopy:

Glomerulus: The capillaries were full of crowded red blood corpuscles, possibly an evidence of haemoconcentration.

Proximal tubule:

A. Pars convoluta: Normal appearances. Normally thin basement membrane.

B. Pars recta: The very thick basement membrane which was observed after hydration, has come back to normal, though incompletely, it still remained slightly thicker than the basement membrane of the thick ascending limb of the loop of Henle (Fig. 203).

Thin segment: The lumina of the thin segments were narrow. The thick

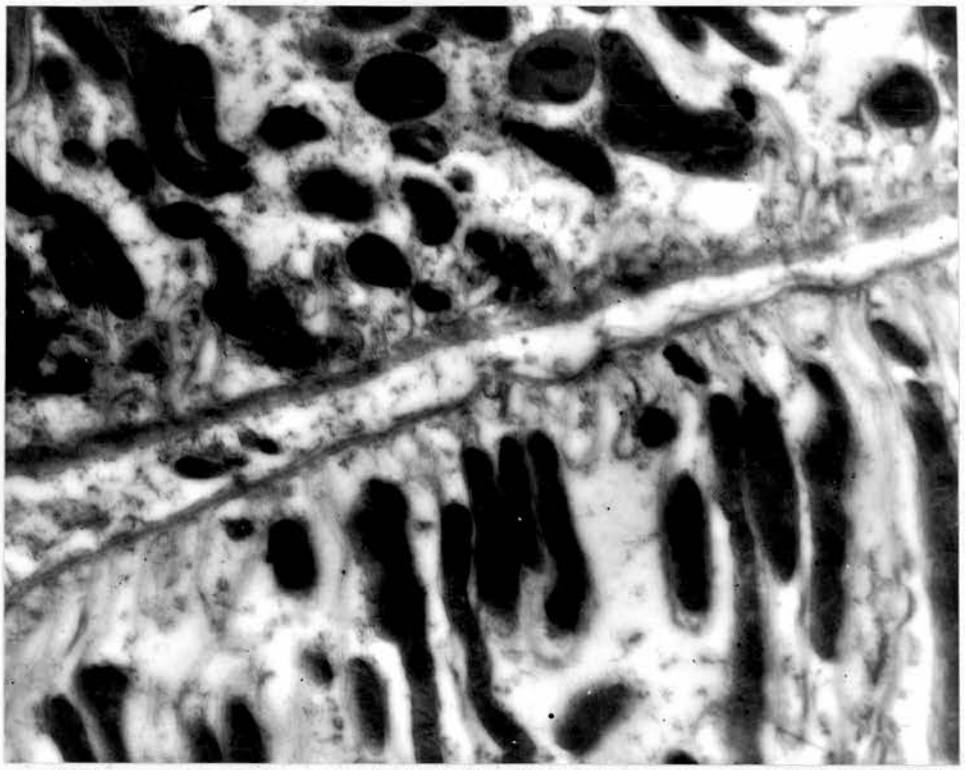


Fig. 203. Hydration followed by dehydration experiment. Rat 7. The basal parts of pars recta of a proximal and a distal tubule. Note that the basement membrane of the proximal tubule (top) is thicker than that of the thick segment of the ascending limb of Henle's loop. x 15,000

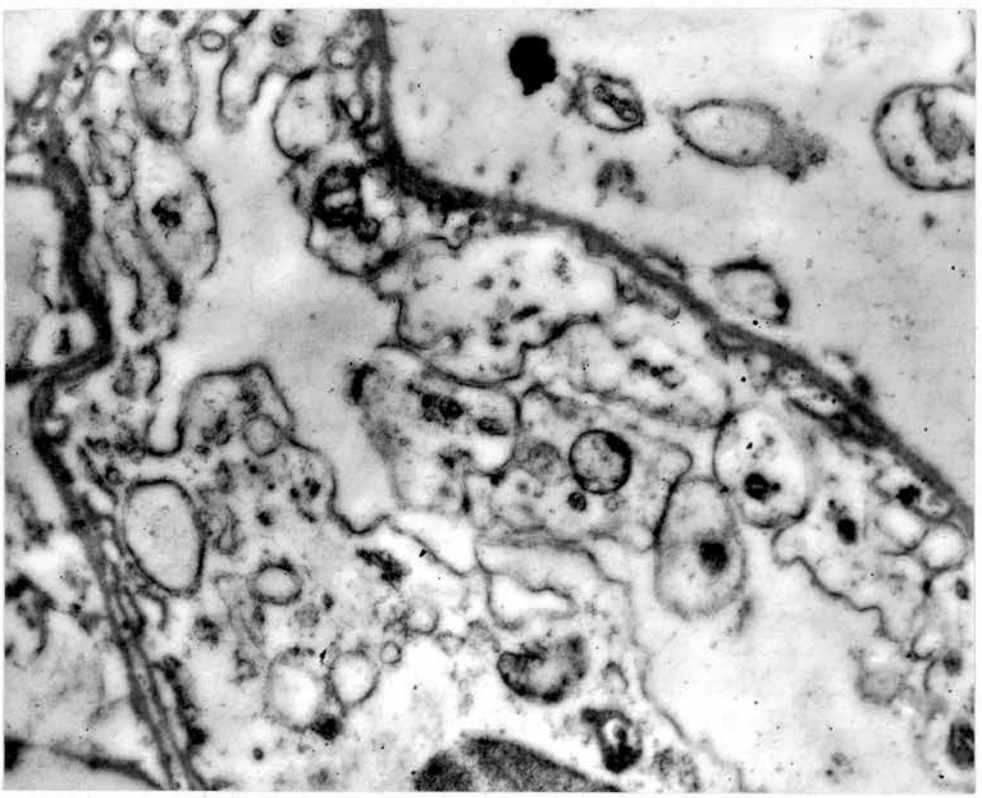


Fig. 204. Hydration followed by dehydration experiment. Thin segment of a loop of Henle. Note the thickness of the basement membrane and the cytoplasmic vacuoles. x 15,000

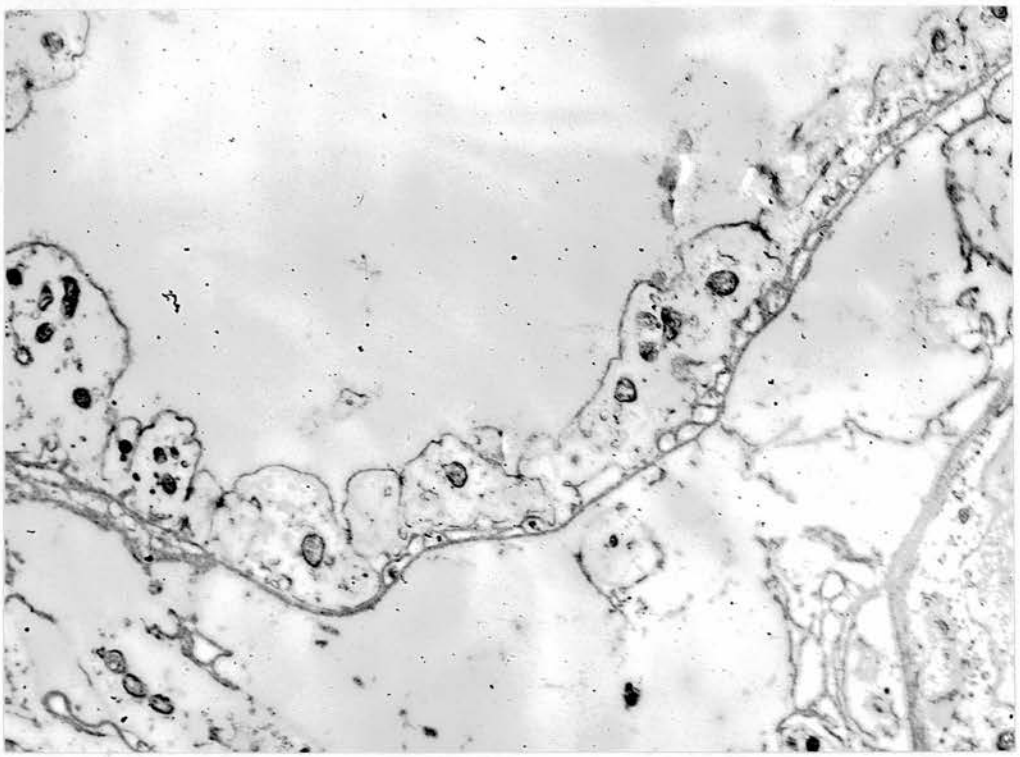


Fig. 205. Hydration followed by dehydration experiment. Rat 8. Thin segment of the loop of Henle in the inner medulla.
x 8,000

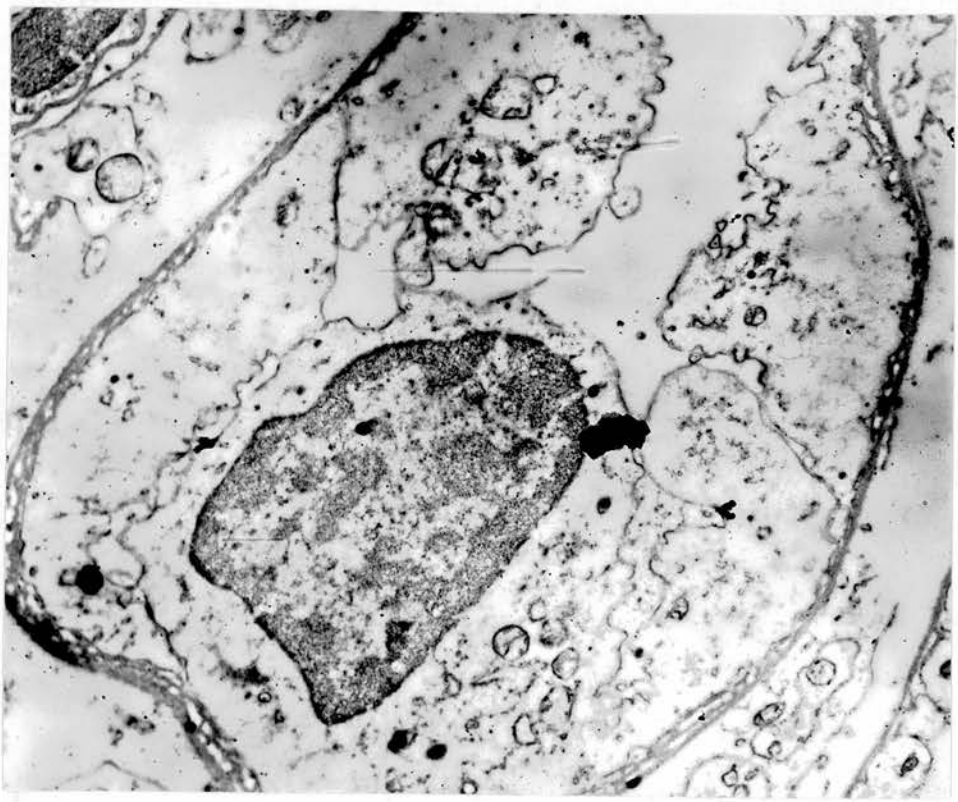


Fig. 206. Hydration followed by dehydration experiment. Rat 9. Thin segment of the loop of Henle in the inner medulla.
x 8,000

Fig. 207. Hydration followed by dehydration experiment: Rat 7. An inner medullary thin segment. Note the increased number of the cell foldings, the numerous cytoplasmic vacuoles and the mottled appearance of the nuclei. x 6,000

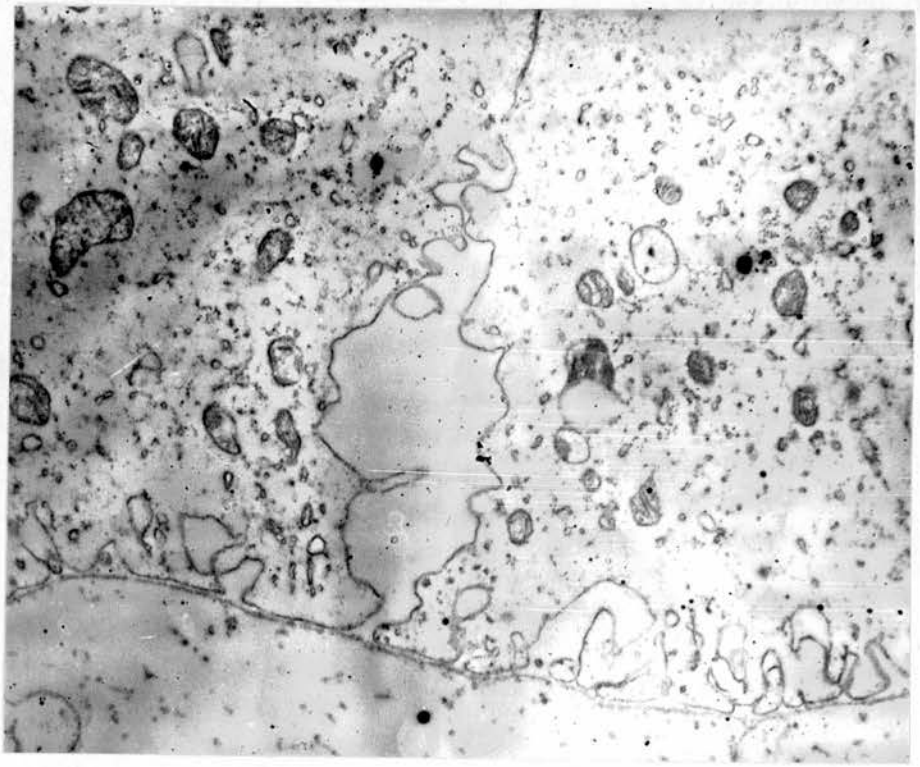


Fig. 208. Hydration followed by dehydration experiment. Rat 9. Two adjacent cells in an inner medullary collecting tubule. Note the partial lateral separation, the intact terminal bar and the cytoplasmic vacuoles related to the mitochondria. x

basement membrane observed in the descending thin segments after hydration was found to have largely regressed after dehydration (Fig. 204). However, this regression in thickness was incomplete, particularly in the two rats that received 20 ml. water (compare the thickness of the basement membrane in Fig. 205, presumably of the thin ascending limb to that if Fig. 206, presumably of the thin descending limb).

Another noticeable feature that appeared in most thin segments is the increased number of the basal cell foldings and the large number of vacuoles that became apparent within the cytoplasm (Fig. 204 and 207).

Thick segment and distal convoluted tubule: Were normal.

Collecting tubule: The rat that received 5 ml. had normal collecting tubules. In the other two rats, a slight degree of cell separation was observed between the light cells in the inner medullary zone. This was never complete, the terminal bars were always intact and no bare areas of basement membrane were seen (Fig. 208). A few granulated vacuoles were occasionally seen in the light cells in the inner medulla (Fig. 208). Nuclear changes similar to those observed in the dehydration experiment (vide infra) were also seen.

Capillaries: The thickened basement membrane of the descending vasae rectae capillaries has largely, though incompletely regressed.

III. Dehydration experiment:

The urine osmolality at the end of the dehydration period is seen in Table (7).

Table 7.

Rat No.	Weight g	Duration of dehydration	Urine Osmolality m.Osm/Kg.
10	400	24	2750
11	400	48	2900
12	300	48	2970
13	320	48	3000

Oh light microscopy: No abnormality was detected.

On Electron microscopy:

The capillaries in the glomerulus, cortex, the outer medulla and the inner medulla were very full of crowded red blood corpuscles (Fig. 211 and 218) indicating haemoconcentration.

The nuclei of all the cells in the medulla, particularly in the inner medulla showed a striking mottling with moth-eaten appearance of the chromatin (Fig. 209).

Proximal tubule: Both the pars convoluta and the pars recta were normal. In particular, the basement membrane was normally thin (Fig. 210).

Thin segment: All the thin segments showed the same appearance whether they were in the outer or in the inner medulla. The lumen was usually narrow, sometimes very narrow (Fig. 211). The basement membrane was normally thin (Fig. 212). The basal cell foldings were very complex, very deep and very numerous (Fig. 212 and 213). The luminal surface was deeply indented by a number of deep complex channels (Fig. 214 and 215). Papilliform projections were frequently seen arising from the luminal surface (Fig. 216 and 217) and these, together with the deep crypts greatly increased the

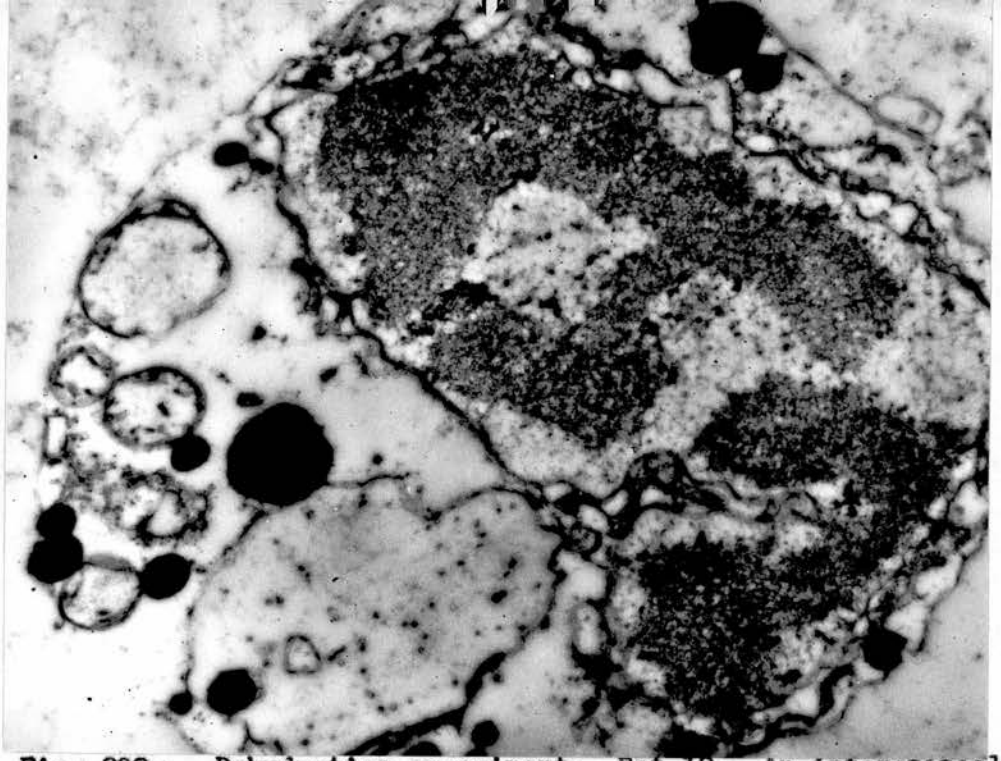


Fig. 209. Dehydration experiment. Rat 10. An interstitial cell in the inner medulla showing a striking mottling of the nuclear chromatin. x 12,000

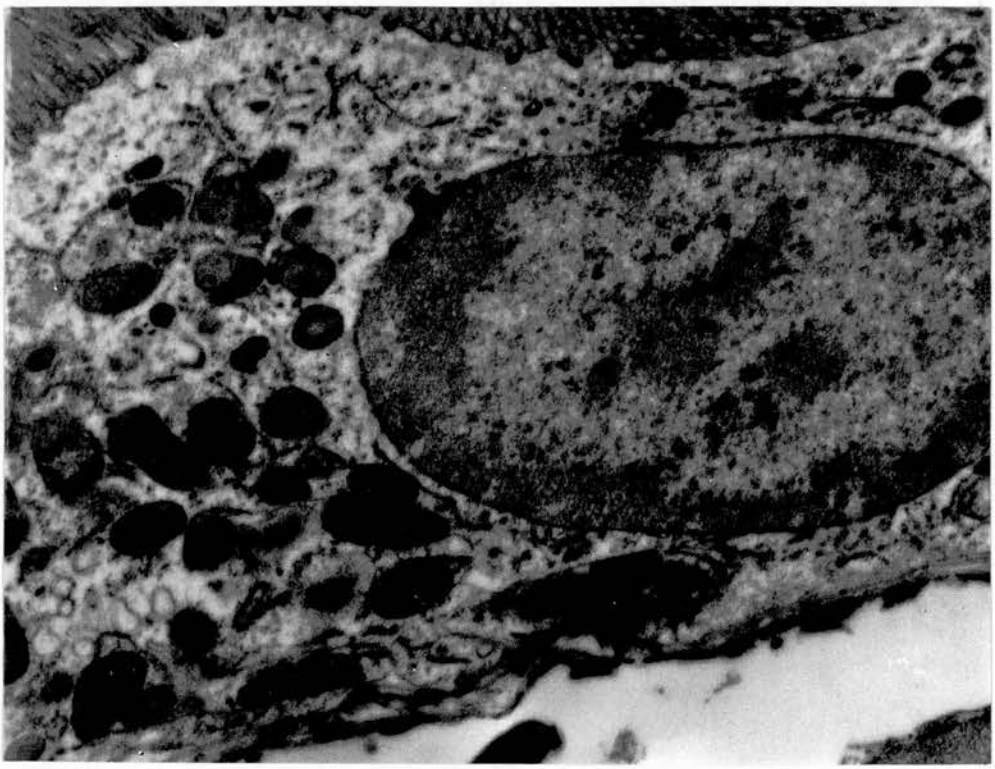


Fig. 210. Dehydration experiment. Rat 13. Pars recta of a proximal tubule. Note the thickness of the basement membrane and compare with Fig. 164. x 15,000

Fig. 211. Dehydration experiment: Rat 10. Thin segment of a loop of Henle in the outer medulla. The lumen is very narrow, the luminal and the basal cell foldings are very numerous and the capillaries are crowded with red corpuscles. x 2,500

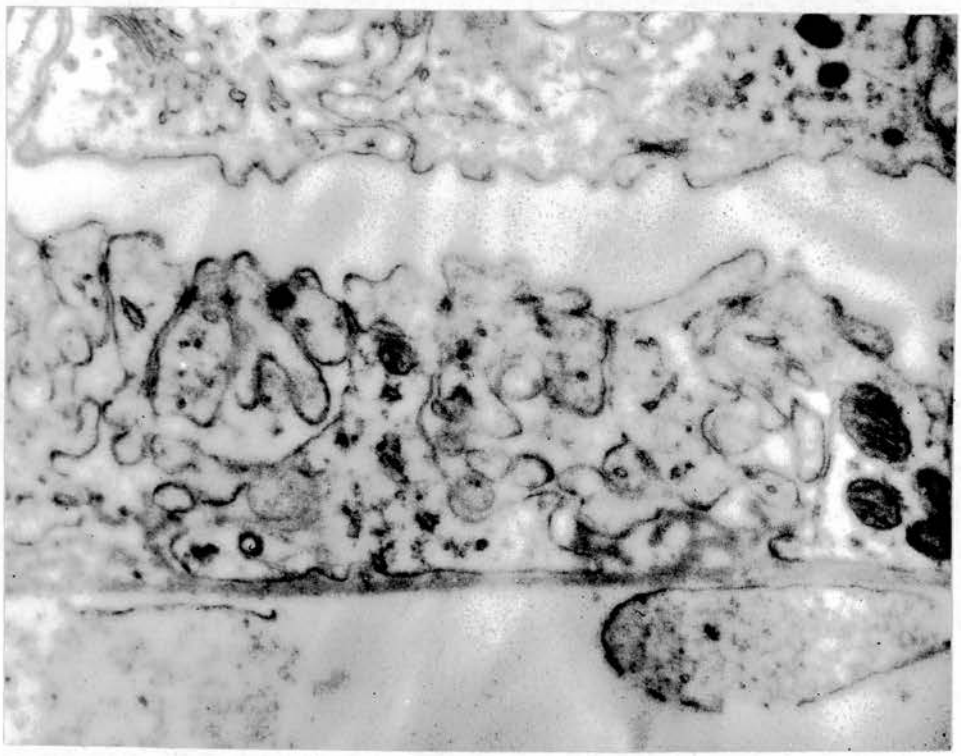


Fig. 212. Dehydration experiment: Rat 10. Thin segment of a loop of Henle in the outer medulla. The basement membrane is of normal thickness and the basal cell foldings are very numerous, deep and complex. x 24,000

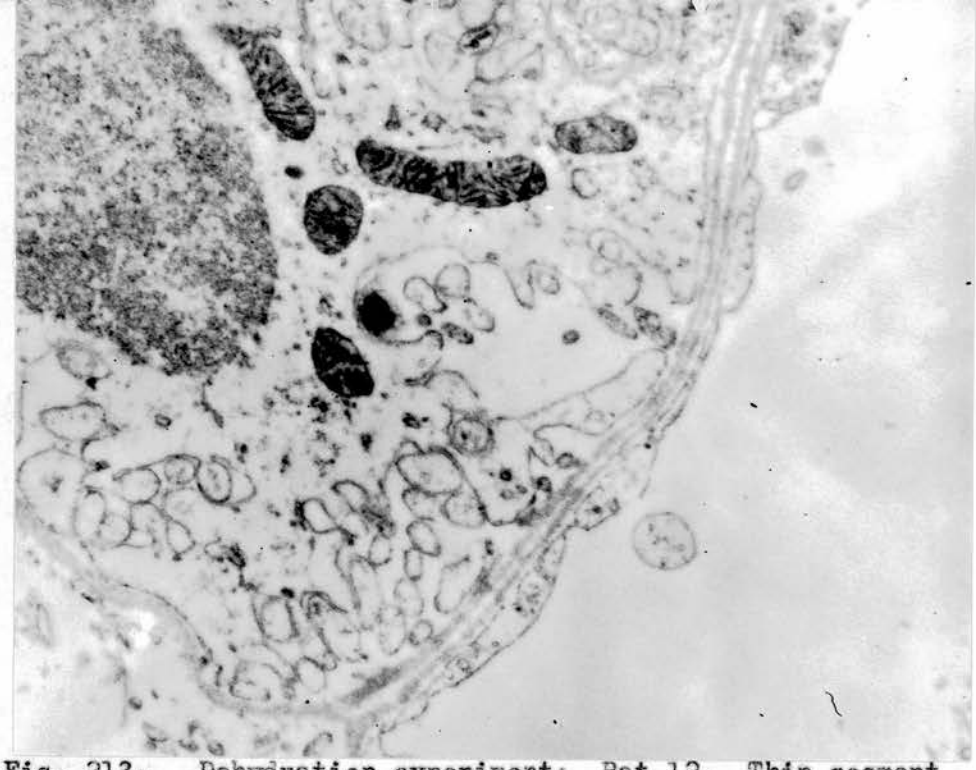


Fig. 213. Dehydration experiment: Rat 12. Thin segment of a loop of Henle in the outer medulla. Note the numerous complex basal cell foldings. x 15,000

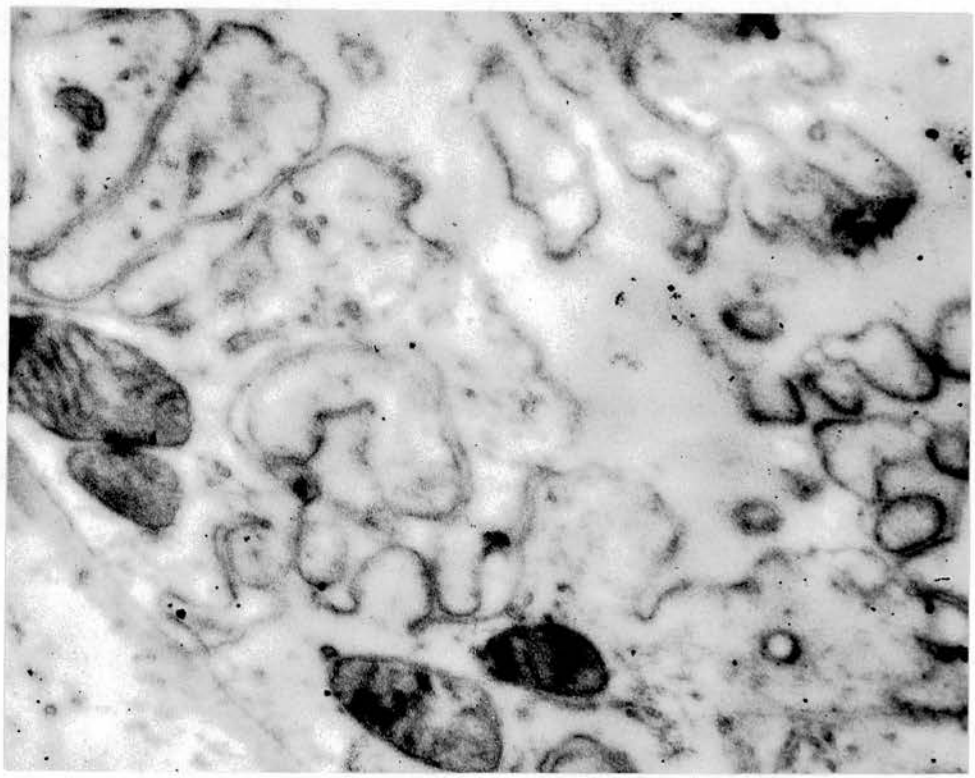


Fig. 214. Dehydration experiment: Rat 12. Thin segment of a loop of Henle in the outer medulla. Compare the appearance and the thickness of the basement membrane with that in Fig. 173. Note the channels running from the lumen deeply into the cytoplasm. x 30,000

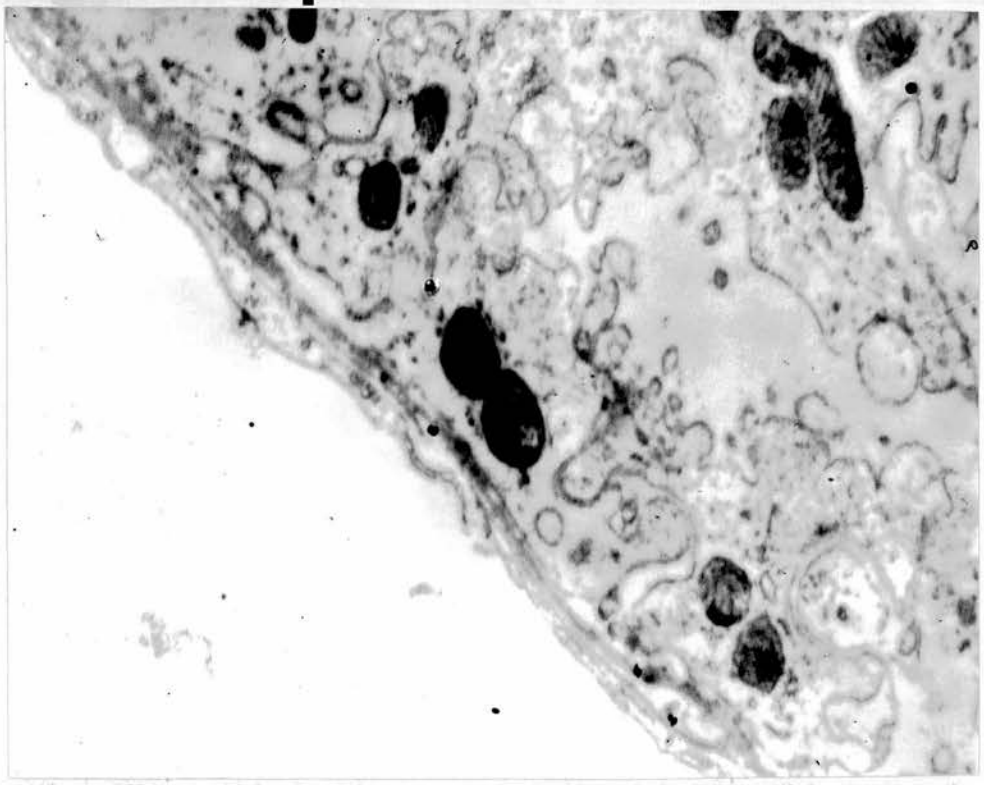


Fig. 215. Dehydration experiment: Rat 12. Thin segment of a loop of Henle and an adjacent efferent capillary in the outer medulla. Note the complex deep channels running from the tubular lumen into the cells. x 19,500

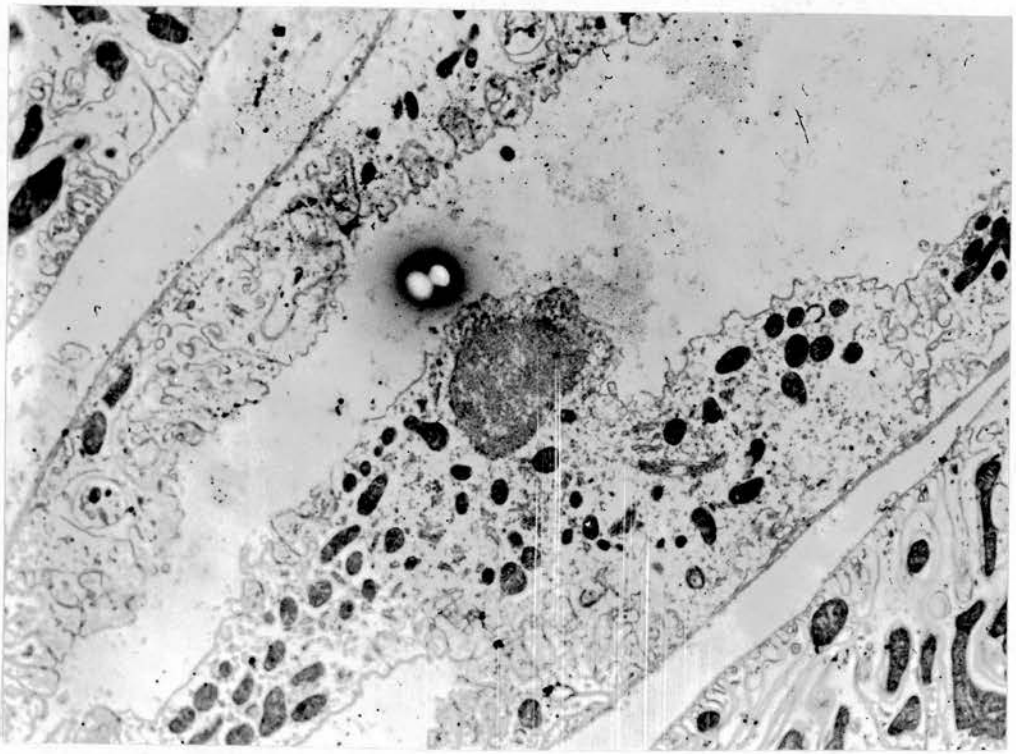


Fig. 216. Dehydration experiment: Rat 12. Thin segment of a loop of Henle in the outer medulla. Note the papilliform process projecting into the lumen. x 6,000

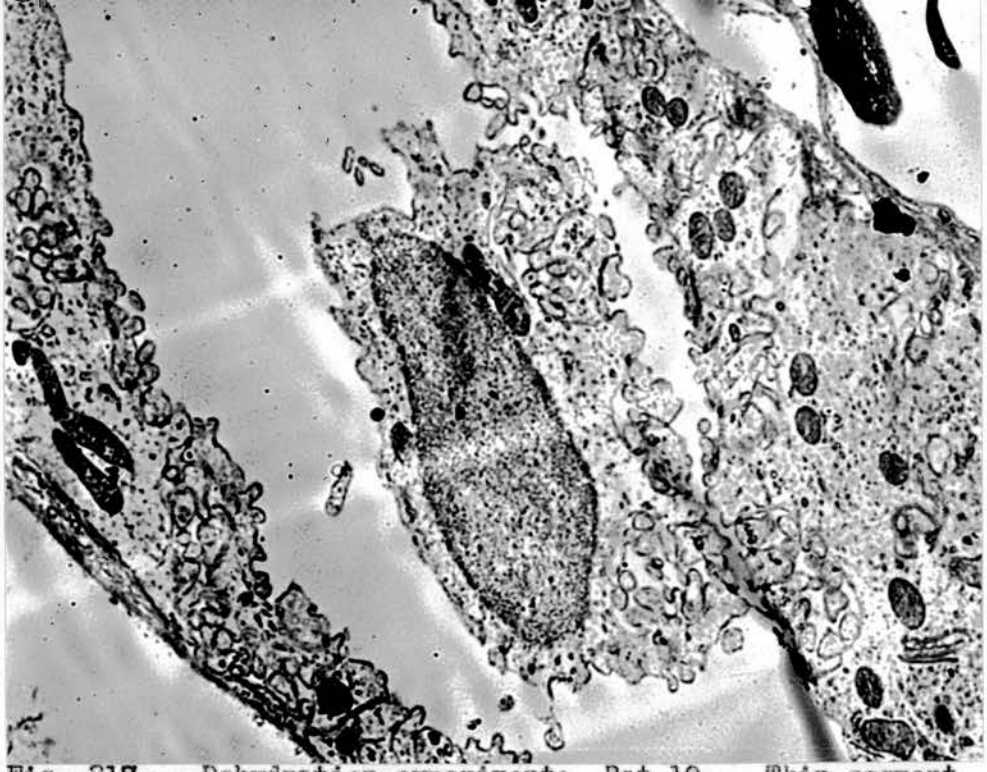


Fig. 217. Dehydration experiment: Rat 10. Thin segment of a loop of Henle in the inner medulla. Note the numerous luminal cell foldings and the papilliform projection into the lumen. x 9,000

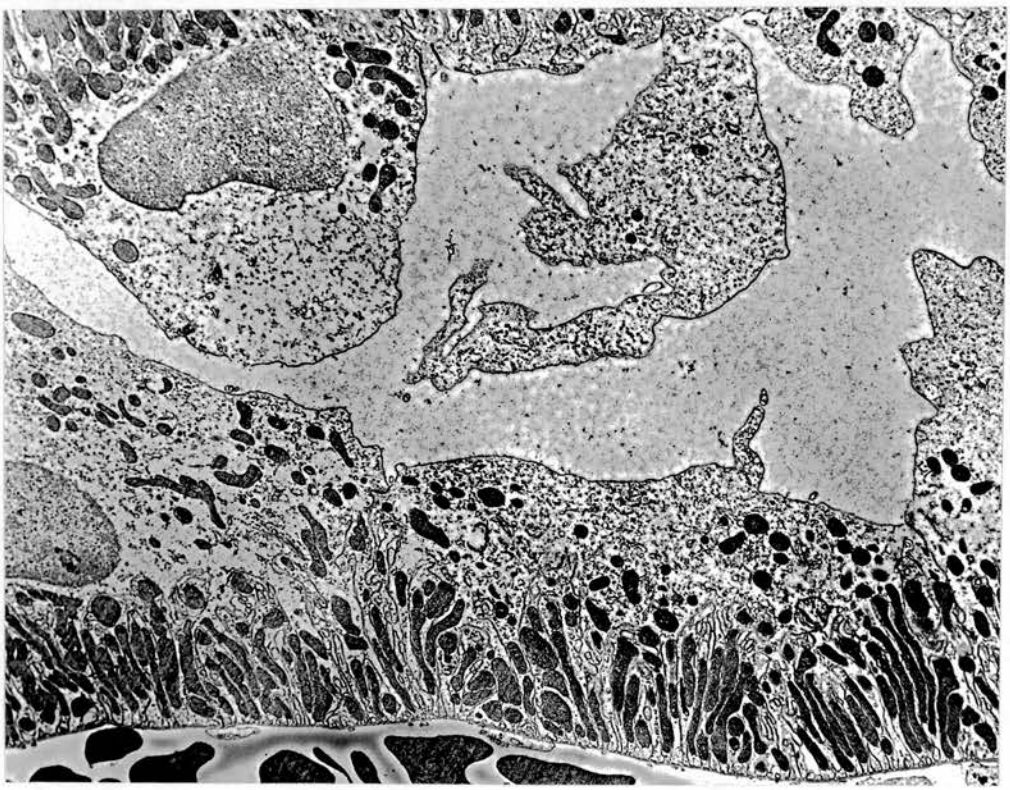


Fig. 218. Dehydration experiment: Rat 10. Thick segment of a loop of Henle. x 2,500

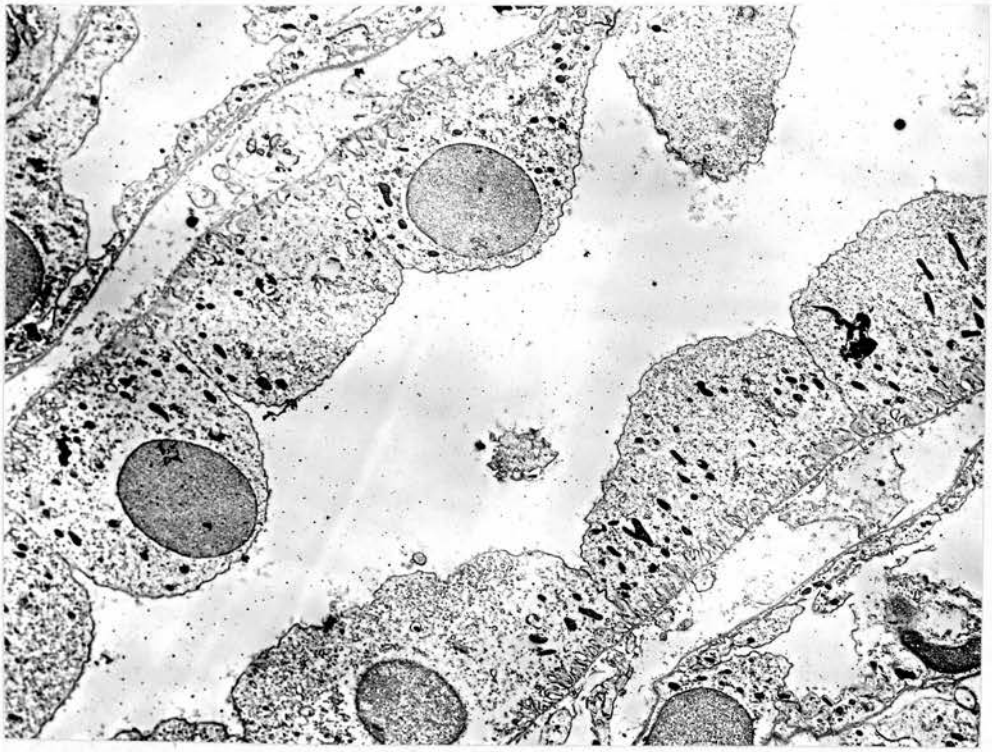


Fig. 219. Dehydration experiment: Rat 13. An inner medullary collecting tubule. x 1,500

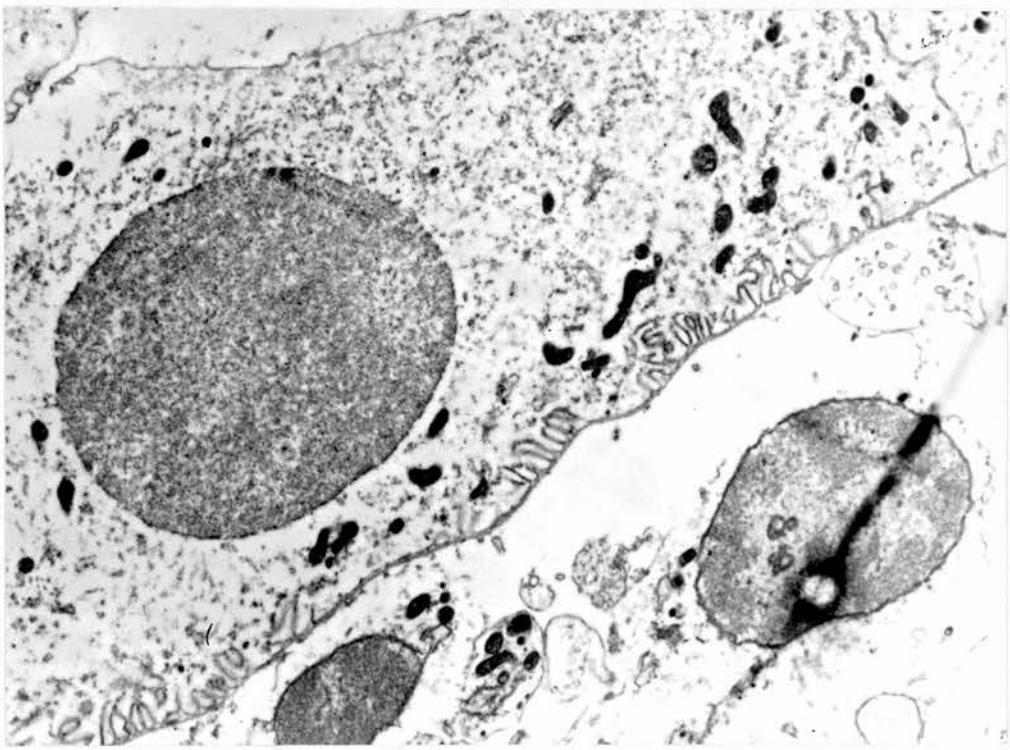


Fig. 220. Dehydration experiment: Rat 12. A light cell of a medullary collecting tubule. x 6,000

luminal cell surface in the same way as the deep, numerous, complex basal foldings greatly increased the basal cell surface. The cell cytoplasm was full of a moderate number of vacuoles with clear contents.

Thick segment and distal convoluted tubule: Were normal (Fig. 218).

Collecting tubule: The collecting tubules appeared quite normal. Lateral cell separation was never encountered (Fig. 219). The light cells showed no vacules or granuoles (Fig. 220) and the dark cells did not show any increase in the basal or luminal cell surfaces (Fig. 221), as was seen in the collected tubules in the hydrated rats.

Capillaries: Both types of capillary were normal; in particular, the basement membrane of the descending thick capillaries was normally thin.

IV. Pitressin experiment:

The urine osmolality and the duration since the administration of pitressin are given in Table (8).

Table 8.

Rat No.	Weight g.	Dose of pitressin given I/V.milli-units.	Duration since administration of pitressin,minutes.	Urine Osmolality M.Osm./Kg.
14	280	50	30	2280
15	250	50	45	2600
16	250	50	60	2400

On light microscopy: No abnormality was detected.

On Electron microscopy:

The findings in this experiment were very similar to those found in the dehydration experiment with two important exceptions.

(1) No evidence of haemoconcentration was seen.

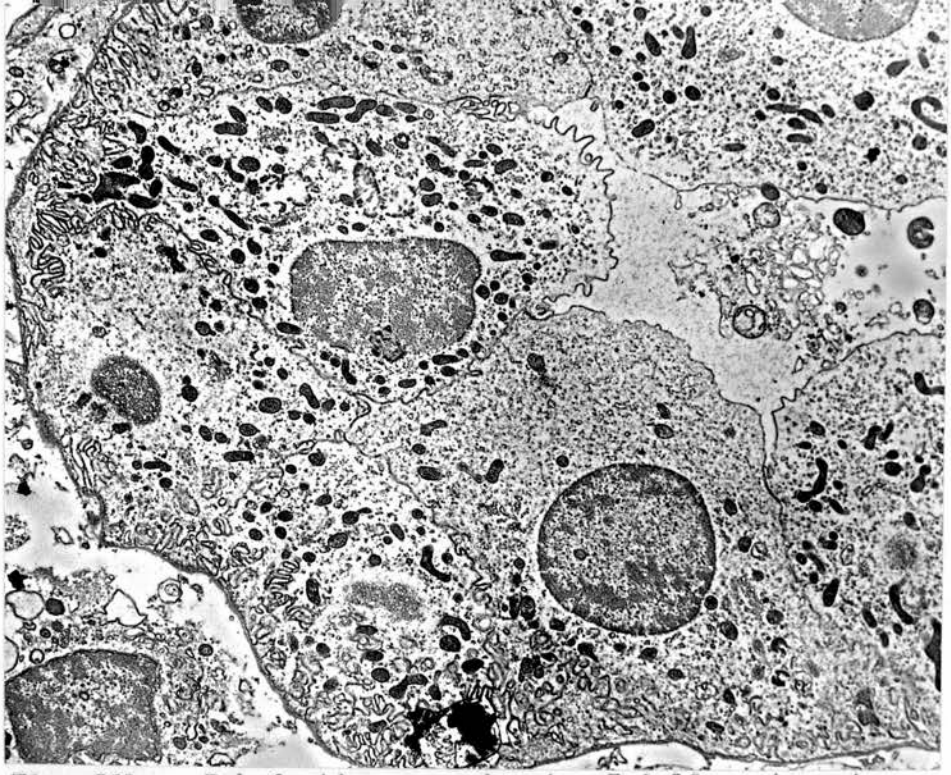


Fig. 221. Dehydration experiment: Rat 10. An outer medullary collecting tubule. x 2,500

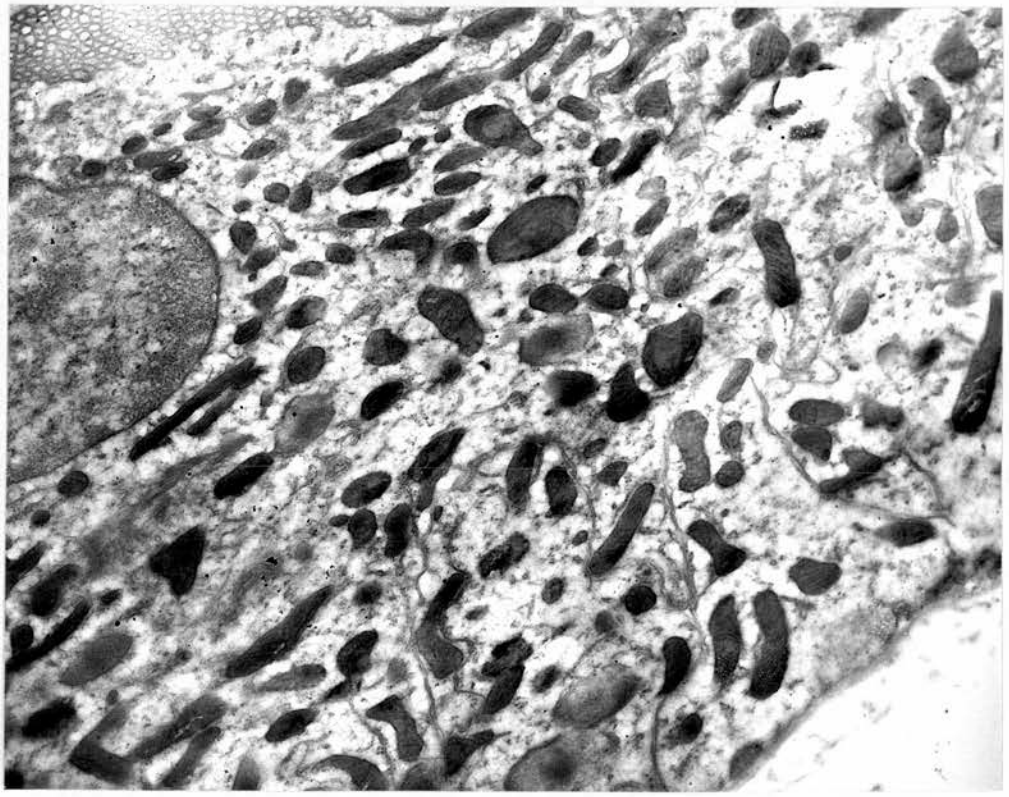


Fig. 222. Pitressin experiment: Rat 15. Pars recta of a proximal rubule. The lumen is closed by densely packed microvilli and the basement membrane is thin. x 12,000

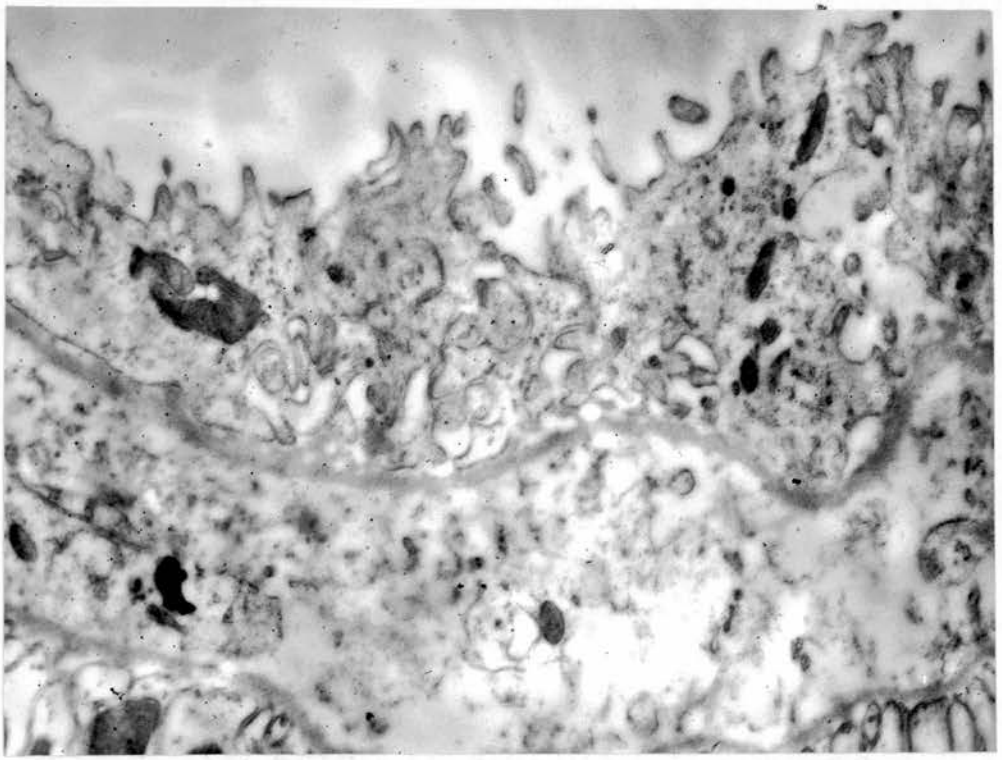


Fig. 223. Pitressin experiment: Rat 15. An outer medullary thin segment. The basement membrane is of normal thickness, and the luminal and basal cell membranes show numerous complex foldings. x 15,000

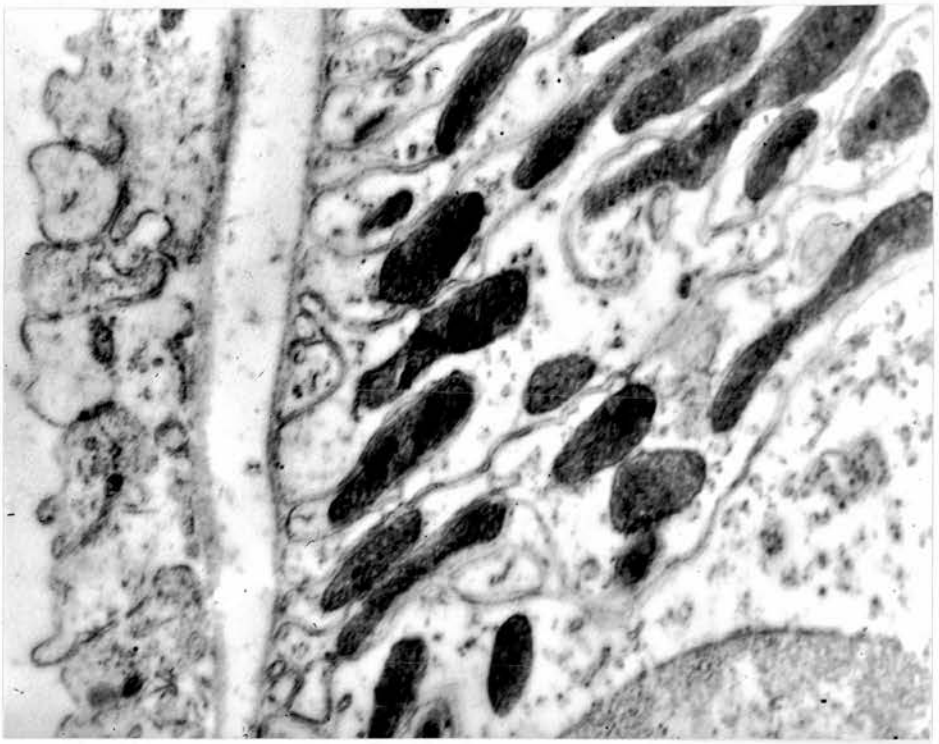


Fig. 224. Pitressin experiment: Rat 14. The basement membrane of the thin segment of the loop of Henle is as thin as that of the adjacent thick segment. x 24,000

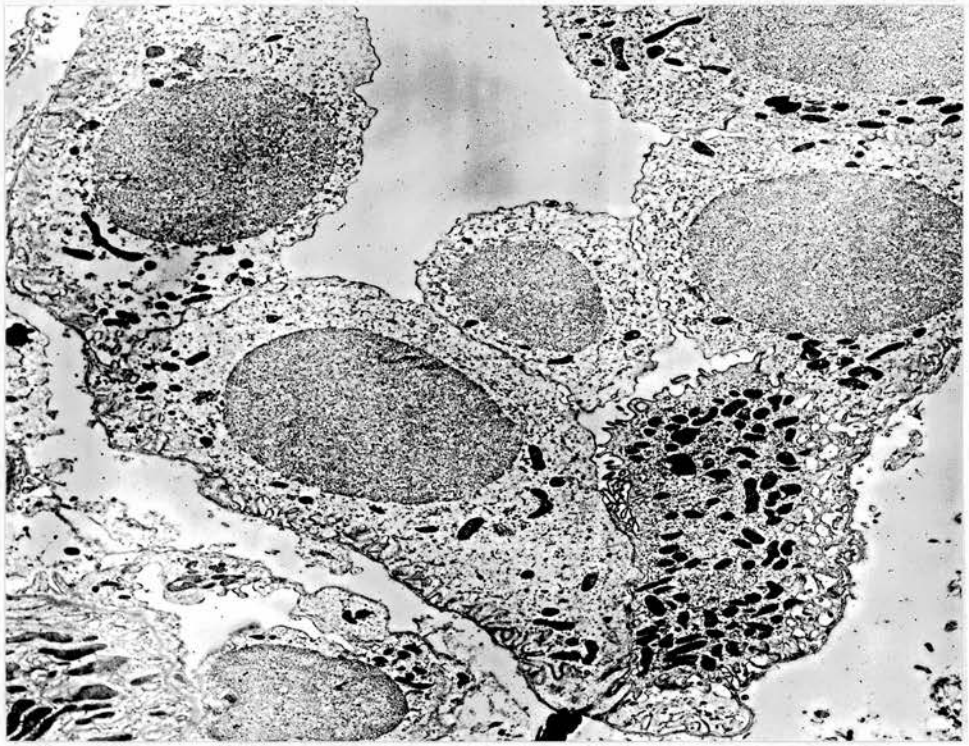


Fig. 225. Pitressin experiment: Rat 15. An outer medullary collecting tubule. x 4,000

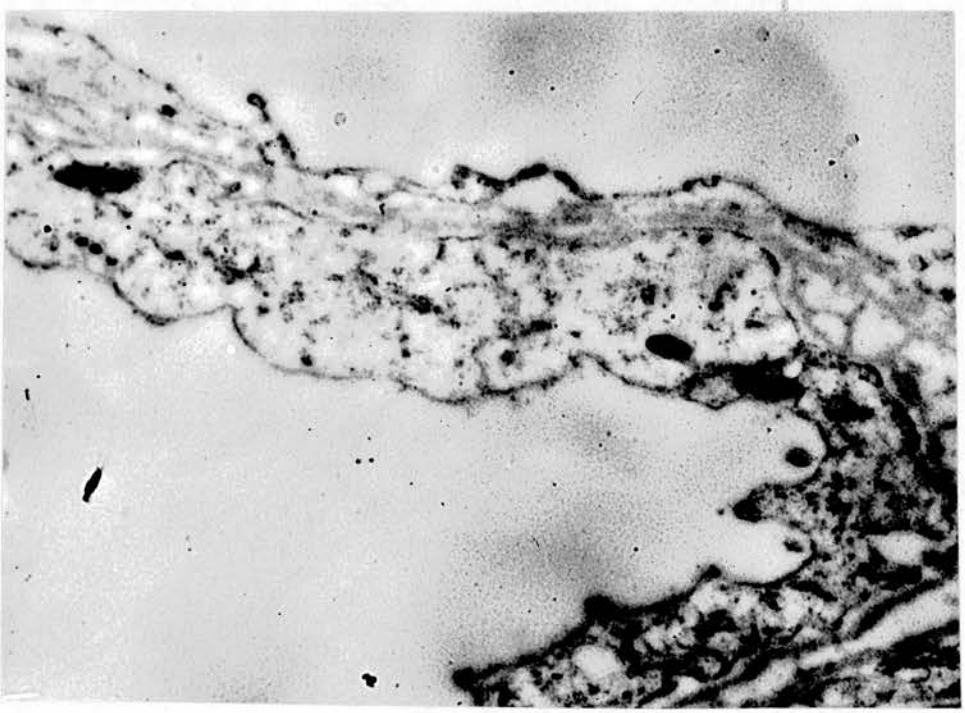


Fig. 226. Pitressin experiment. Rat 15. An efferent (top) and an afferent capillary in the outer medulla. Note the normal thickness of the basement membrane and compare with Fig. 198 and 199, which are of the same magnification. x 24,000

(2) no mottling of the chromatin of the cell nuclei in the medulla was observed.

The pars recta of the proximal tubule had a normally thin basement membrane (Fig. 222).

The thin segments in the outer as well as in the inner zones of the medulla showed numerous, complex, deep folding of both the luminal and the basal cell membranes (Fig. 223). The basement membrane of all the thin segments was normally thin (Fig. 224). However, the lumina of the thin segments were not particularly narrow as in the dehydrated rats.

The collecting tubules were normal (Fig. 225). No lateral separation between the cells was noticed. The light cells showed no vacuoles or granules in their cytoplasm and the dark cells did not show an abnormal increase in the basal or luminal cell foldings (Fig. 225).

The capillaries were entirely normal. In particular, the basement membrane of the descending vasae rectae capillaries was normally thin (Fig. 226).

DISCUSSION.

It has long been recognised that in the mammalian kidney, the antidiuretic hormone of the neurohypophysis must have an action on the renal tubule which results in enhanced reabsorption of water, so that the osmotic pressure of the urine is raised above that of the plasma. With appropriate doses of the hormone, full antidiuresis is obtained without significantly altering filtration rate. Some modifications of solute reabsorption may ensue, but the increased urine concentration is due in the first place to the greatly diminished urine volume.

Oddly enough, in the absence of antidiuretic hormone the kidney is not producing what would seem to be the easiest thing to produce. An isotonic urine, in a normal kidney, is the result of a moderate antidiuretic action. With no antidiuretic hormone circulating in the body, as in water diuresis, urinary osmotic pressure is reduced well below isotonicity. If therefore we want to approach the antidiuretic mechanism, we ought to start at the bottom of the problem, investigating the condition of water diuresis first.

Among the recent investigators, only two have tried to use changes in the morphology of the nephron for the study of the mechanisms of concentration and dilution of the urine and the mode and site of action of the antidiuretic hormone. The first study was that reported by Ginetsinskii in 1958 (12). This author, studied by the light microscope, the kidneys of forcibly hydrated, and dehydrated rats as well as the kidneys of rats after a dose of ADH. From his findings, he proposed that the action of the antidiuretic hormone is to cause an apocrine secretion of hyaberonidase by the collecting tubules. The released enzyme was then thought to hydrolyse the hyaberonic acid in between the collecting tubule epithelial cells, thus creating intercellular "pores" and rendering the tubule more permeable for the back diffusion of water in conditions of dehydration or under the influence of administered ADH. The results reported in this thesis utilising electron microscopic techniques show that Ginetsinskii's findings are completely fallacious. Lateral separation of collecting tubule cells was clearly noticed after hydration and were never observed in the dehydration or pitressin experiments. When one studies his published photomicrographs (12) their poor quality becomes immediately noticeable and can easily explain that

his findings were artefacts. Moreover, the stain he used, toluidine blue, and considered to be specific for hyaluronic acid, is not specific in any way and he himself admits this and says in a later publication "..... there is no histochemical reaction specific for hyaluronic acid" (13). Finally, Leaf (30) has recently reported that when commercial hyaluronidase, even in large amounts, was added to the medium bathing both surfaces of the isolated toad bladder (an experimental preparation which mimics the activities of the mammalian renal tubule in this respect), it produced no detectable effects on the permeability of water, whereas subsequent addition of pitressin still reproduced its characteristic effects. This indicated that the effects of the antidiuretic hormone are not mediated through an action of hyaluronidase.

The second morphological study was that reported in 1960 by Lapp (27). This author studied by the electron microscope the renal medulla in rats in water diuresis and in short and long dehydration experiments; He did not report any alterations in the loops of Henle or in the blood capillaries in these conditions; he only described changes in the collecting tubules. He reported that the dark intercalated cells of the collecting tubules bulge for a considerable distance in the lumen in both the diluting and the concentrating kidney. Moreover, in the concentrating kidney, he observed vacuolar changes in the mitochondria of these cells. In the studies reported in this thesis bulging of the dark cells with papilliform projections into the lumen was found only in water diuresis, while the dark cells and their mitochondria appeared quite normal in the concentrating kidney. Lapp also described dislocation and lateral separation of the light cells on the basement membrane side in water diuresis. This has been confirmed and it was shown in addition that the lateral separation between the cells is complete in

many places leaving bare areas of basement membrane.

The most striking and the most important changes found in this study have not been described before. The marked thickening of the basement membrane of the descending limb of the loop of Henle, in water diuresis, whether this limb is the pars recta of the proximal tubule or the thin descending segment, is an entirely new discovery. On the other hand, this basement membrane was found to be very thin, as thin as the basement membrane of the rest of the nephron, in the dehydrated animals and in animals to whom ADH has been administered.

The basement membrane is a condensed layer of the ground substance and may be related to it physically and chemically. Physically the ground substance is a gel or a sol, of varying consistency, composed of salts and water with proteins and polysaccharides in solution. Six different kinds of mucopolysaccharides have been distinguished in the ground substance from various tissues: hyaluronic acid, three varieties of chondroitin sulphate, chondroitin alone and keratosulphate (35). Little is known about the chemical composition of the protein of the ground substance. The physical state of this ground substance, or of the basement membrane, however, appears to be related to the degree of polymerisation of the polysaccharide complexes (11). The ground substance and the tissue fluid are homogeneous and interconnected, so that the consistency of the ground substance (and possibly basement membrane) varies from a gel-like to a more watery state (35).

Beyond these general features, very little is known about the precise chemical nature of basement membranes, their permeability characteristics and their other physical properties. Nobody has even suggested that the

basement membrane might have different chemical composition and different functions in the different parts of the body.

As far as the basement membrane of the nephron is concerned, I feel that it differs chemically from one part to another. The basement membrane of the ascending limb of the loop of Henle has been considered to be permeable to Na^+ and impermeable to water, while the basement membrane of the descending limb is considered permeable to water (17,64). The behaviour of the basement membranes of these two limbs to the same insult, a water load, has been found in this study to be very different. Such differences in permeability properties and in the reactions to the same factor must indicate that they are different in their minute chemical composition. A difference in chemical composition will impart different physical and permeability properties to the two limbs of the loop and I think that it is this change in the composition of the basement membrane of the loop at the bend, rather than a difference in function of morphologically similar simple squamous epithelial cells in the descending and ascending limbs of Henle's loop, that allows this part of the nephron to function as a countercurrent multiplier system. Moreover, any alteration in the physical state of the basement membrane of one of the limbs, leading to a loss in the differences of permeability between the two limbs, will inevitably result in nullifying the functional accomplishment of the loop as a countercurrent multiplier.

From the data obtained by micropuncture, microanalysis and microcryscopy techniques, one can propose numerous variations of the countercurrent hypothesis that will explain the facts that are now available. However, the electron microscopic studies reported here, have shown that the main morphological difference in the nephron between water diuresis and dehydration

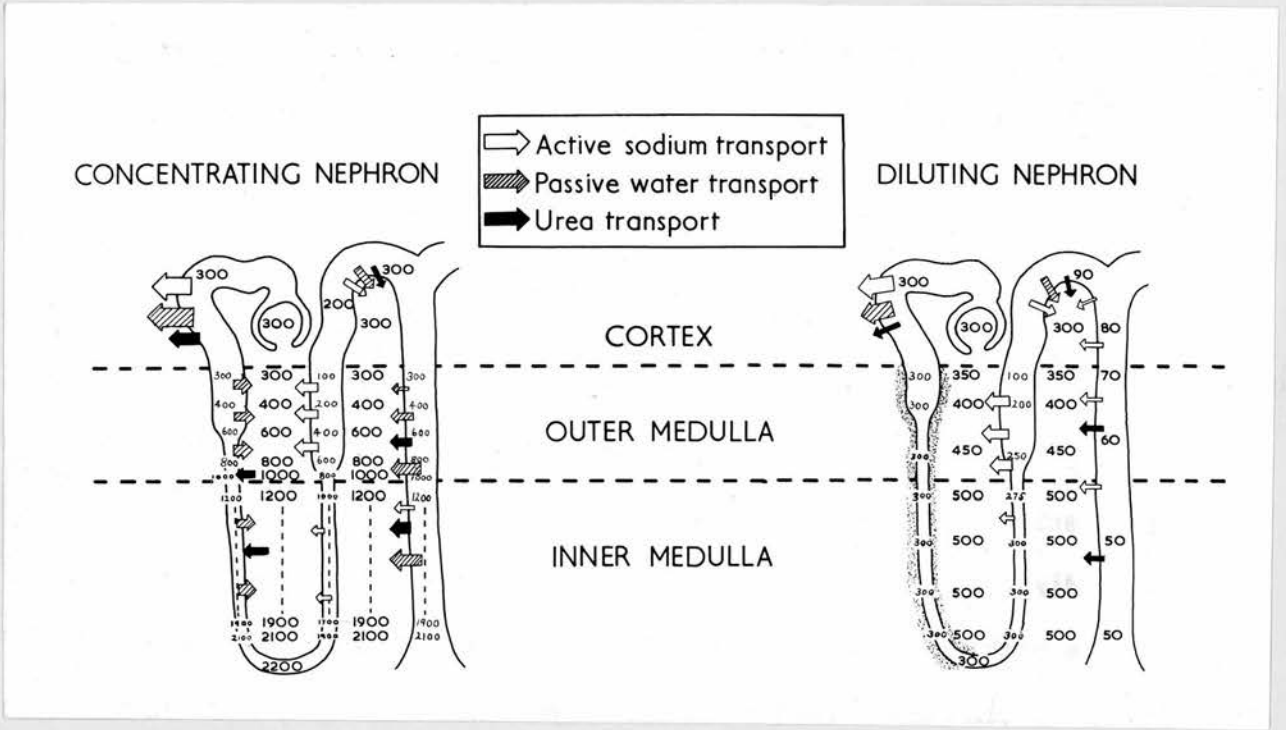


Diagram 4.

Diagram representing the proposed hypothesis explaining the mechanisms of urinary concentration and dilution. The numbers represent hypothetical osmolality values. A semiquantitative significance is attempted in the number and thickness of the arrows. Note that the state of the concentrating nephron is similar to that seen in the accepted version of the countercurrent hypothesis. In the new hypothesis the descending limb of the loop of Henle is considered to be impermeable to water (and possibly to urea) in the absence of the antidiuretic hormone and therefore the loop does not function as a countercurrent multiplier in the diluting nephron.

is in the basement membrane of the descending limb of the loop of Henle. This very important new piece of information must be taken into consideration in any hypothesis trying to explain the function of urinary concentration or dilution. There is nothing in these results to disagree with the suggestion that the loop of Henle functions as a countercurrent multiplier system and that the active transport on which the system depends is performed by the nephron. These results, however, must modify the current views. The modified working hypothesis proposed is as follows: Diagram (4).

"Sodium, by an unknown active mechanism, and chloride, as a result of the electro-chemical gradient established, are transported out of the relatively water impermeable ascending limb of the loop of Henle into the interstitium of the medulla until a gradient of perhaps 170 - 200 mOsm/Kg of water is established between the fluid of the ascending limb and the interstitium. This sodium pump is situated all along the length of the ascending limb; active sodium transport being a function of all the cells of the nephron. However, the much more complex internal structure of the cells of the thick segment of the ascending limb makes this segment much more efficient in Na^+ transport than the thin segment. This single effect is multiplied as the fluid in the descending limb, in conditions of dehydration in presence of the antidiuretic hormone, comes into osmotic equilibrium with the interstitial fluid by the diffusion of water out of the descending limb, thus raising the osmolality of the fluid presented to the ascending limb. In this fashion, an increasing osmotic gradient is established in the direction of the tip of the papilla, and yet at no level is there a large osmotic difference between luminal and interstitial fluid. The epithelium and basement membrane of the collecting ducts, in this scheme, are always permeable to water. This results in diffusion of

water out of the collecting ducts into the hyperosmotic medullary interstitium until the fluid remaining in the collecting ducts becomes correspondingly concentrated.

In water diuresis, in absence of ADH, a large volume of water passing along the pars recta of the proximal tubule, and the thin descending limb, is attracted to the hyperosmotic interstitium. As the basement membrane of the descending limb is traversed by this large volume of water, it imbibes part of it, holds it intimately, swells and thereby withstands a much higher pressure and hinders the further flow of water out of it. This assumption as to what occurs in the basement membrane of the descending limb of the loop of Henle is taken by analogy of what was proved for structures of a similar composition.

a) Wharton's jelly, a mixture of fine fibrils, polysaccharides, proteins, salts and water; just as basement membranes are found to imbibe water and to hold it intimately in no discernible anatomical structure; a large part of the water is held after cutting up a swollen cord. The swollen jelly withstands temporary compression (10).

b) A similar phenomenon is the "Ranvier bulla" produced by subdermal injection of fluid.

c) Experiments with synthetic mixtures of fibres and hyaluronic acid showed this same phenomenon and it was suggested that any suitable combination of water, a high polymer of open structure and fibres can form a system which imbibes water, then hinders the flow of water out of it and becomes resistant to temporary compression (10).

If the basement membrane of the descending limb of the loop consists of polysaccharides, proteins, salts and water in a gel form in the meshwork

of fine fibrils, the following explanation can be given: water enters the gel because of an osmotic difference. The water interacts with the polysaccharide to form a viscous solution which is distributed throughout the meshwork of fibres, an impermeable structure with mechanical rigidity results; deformation necessitates flow of the very viscous solution and this in turn is prevented by the tangle of fibres. Alternatively, the polysaccharide particles might be sufficiently entangled with each other and with the fibres to prevent appreciable displacement of the polysaccharides when moderate pressure is appl ied or when water tries to diffuse through. The type of polysaccharide in the basement membrane is immaterial. The place of hyaluronic acid (which is the polysaccharide in Wharton's jelly and the polysaccharide used by Fessler in the synthetic mixture) can be taken by any other macromolecule, of the open-type coil of structure having a large hydrodynamic hydration and offering a high resistance both to fluids being moved through it and to it being moved with fluid through a meshwork or set of narrow channels.

Thus, in water diuresis, the changes that occur in the basement membrane of the descending limb of Henle's loop will prevent the outward movement of water from the lumen into the interstitium and therefore will prevent the osmotic equilibrium taking place. The fluid in the descending limb will remain isosmotic to the plasma, as the glomerular filtrate and the loop will no longer multiply the osmotic difference created by the active sodium transport out of the ascending limb. The interstitium at the papilla will only be very slightly hyperosmotic and very little water will back diffuse from the fluid running down the collecting ducts.

The fact that the urine passed out in marked water diuresis is hypo-

osmotic and not isosmotic indicates that there are additional factors in this condition that dilute the urine beyond isotonicity. These factors might be:

1) Further Na^+ transport along the pars convoluta of the distal tubule. In support of this is the finding of Wirz by micropuncture studies that the hypotonicity of the tubular fluid at the beginning of the distal convolution is enhanced throughout the length of that tubule in water diuresis (64).

2) The very rapid flow of a large volume of water along the collecting tubules in water diuresis does not allow sufficient time for the hypotonic contents to equilibrate with the slightly hypertonic medullary interstitium. A very strong evidence for this is the fact that a slightly hypertonic urine could be produced in absence of ADH, during water diuresis, when the renal blood flow and the glomerular filtration had been temporarily reduced, by portal clamping of the renal artery (2, 9). The slowly moving small volume of fluid in the collecting ducts had sufficient time to equilibrate with the slightly hyperosmotic medullary interstitium and a slightly hyperosmotic urine wqs produced.

3) An increased activity by the dark cells of the collecting tubules in active Na^+ (or urea) transport, for which there is an electron microscopic evidence will reduce the tonicity of the outgoing fluid further.

In the concentrating kidney, it is necessary to explain how the water and the sodium which are reabsorbed can be returned to the general circulation. This function is performed by the specialised set of capillaries, the vasae rectae, which provide the circulation for the renal medulla. Their special arrangement in hairpin loops and their minute ultrastructure affording a close similarity to the rete mirabile has been described in the previous chapter. Another interesting and possibly important anatomical feature of the vasae rectae is that they are provided

with anastomotic cross-connections between the two limbs of the loop.

By virtue of their characteristic arrangement, the vasae rectae can operate as countercurrent exchangers. Isosmotic blood enters the vasa recta, and as it encounters the increasingly hyperosmotic medullary interstitium, it gains sodium and loses water, so that at the tips of the vascular loops it is highly concentrated. During ascent of the loop, the blood undergoes a progressive dilution through a gain of water and loss of solute. This countercurrent feature of the vasa recta allows blood to circulate through the renal medulla with minimal dissipation of the hyperosmolality of the interstitium. Their passive nature, however, precludes them from making the mechanism more efficient in the sense that they could "boost" the osmolality of the interstitium to a value higher than that achieved by the loop of Henle alone. The vasae rectae, however, do make the multiplier more effective compared to a comparable system receiving a through-and-through type of blood flow, since the countercurrent exchanger nature of these vessels permits a more complete equilibration of the blood with its environment.

One major factor controlling the extent of equilibration achieved by the blood is the rate of blood flow (3). A low rate of flow would favour more complete equilibration and hence would preserve medullary hypertonicity. A rising rate of blood flow increasingly yields a through-and-through type of flow which could literally wash away the solute and hence dissipate the high osmolalities created by the loop of Henle. In water diuresis the thick basement membrane noticed in this study, in the descending vasae rectae would interfere with their permeability and diminish or abolish their function as countercurrent exchangers.

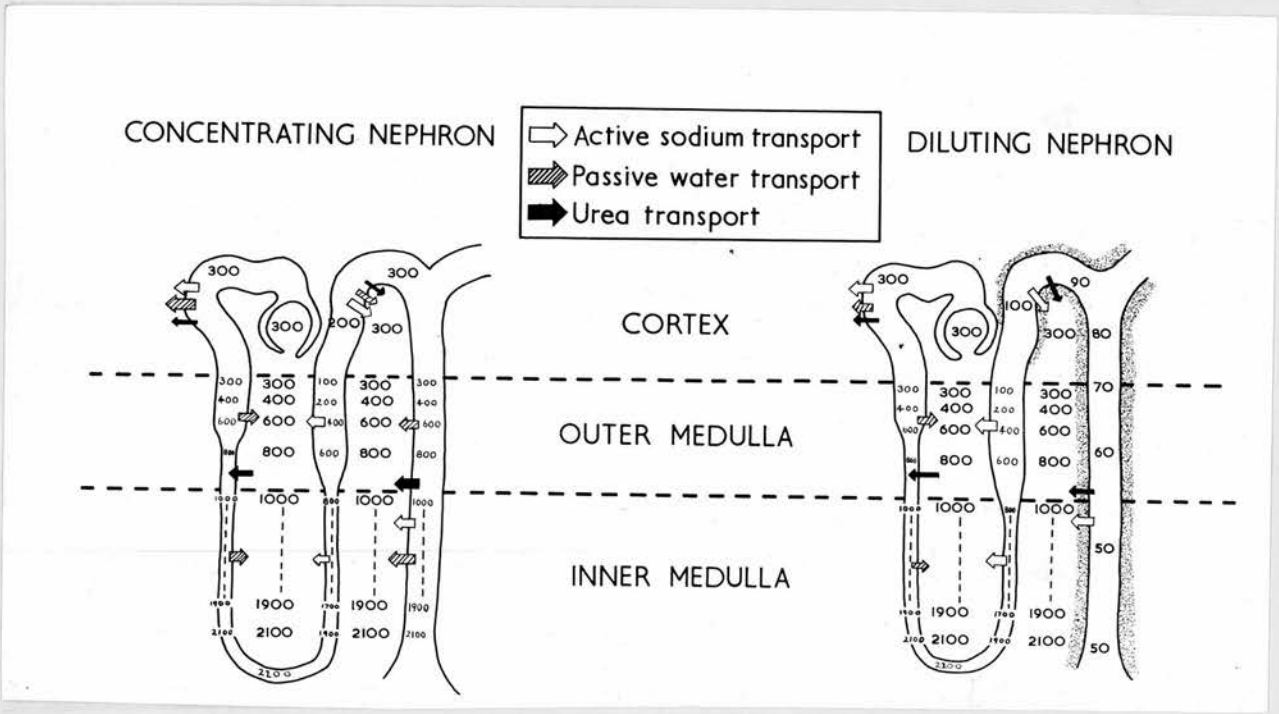


Diagram 5.

Diagram representing the most widely accepted version of the countercurrent mechanism as it is believed to operate in a nephron with a long loop during the processes of concentration and dilution of the urine; this is based largely on the views of Wirz (64) and Gottschalk and Mylle (17). The numbers represent hypothetical osmolality values. No quantitative significance is to be attached to the number or thickness of the arrows and only net movements are indicated. The active sodium transport by the epithelium of the collecting tubule is based on the work of Hilger, Klumper and Ullrich (21) and the urea transport on the work of Lassiter et al (29). Note that in this hypothesis, the distal convoluted and collecting tubules are considered to be impermeable to the passive movement of water in the absence of the antidiuretic hormone while the countercurrent mechanism proceeds equally in both the concentrating and the diluting nephron.

It is virtually certain that the blood in the vasae rectae never achieves complete osmotic equilibration with its surroundings. Indeed, there is a theoretical necessity for assuming that the blood as it leaves the renal medulla is slightly hyperosmotic compared to that which enters the medulla. If this were not the case, the sodium which is reabsorbed by the loop of Henle would not be removed, and the total body sodium would end up in the renal medulla.

The final step in the concentrating kidney involves the removal of water which is reabsorbed from the distal tubule. Conceivably, this occurs by a passive movement of water from the cortical interstitium into the virtually isosmotic blood perfusing the cortex. This movement must dilute that particular blood to some osmolality less than thatof the peripheral plasma. The mixing of this blood with the hyperosmotic blood from the renal medulla leads to a final osmolality for the total renal venous blood which is slightly less than that for the renal arterial blood. This must be the case since, when hyperosmotic urine is formed from isosmotic renal arterial blood, the renal venous blood must be hypo-osmotic."

This modified hypothesis differs from the most acceptable version of the countercurrent hypothesis in the following important points:
(Compare Diagram 4 with Diagram 5).

1) In the hypothesis presented here, the loop of Henle acts as a countercurrent multiplier in the concentrating kidney only. In the currently acceptable hypothesis, the loop of Henle acts as a countercurrent multiplier both in the concentrating and in the diluting kidney.

2) The site of action of the antidiuretic hormone in the current hypothesis is on the distal convoluted and the collecting tubules. In the

suggested here, the site of action of ADH is on the descending limb of the loop of Henle.

3) The mode of action of ADH in the current hypothesis is the opening up of "pores" in the walls of the distal convoluted and collecting tubules. These "pores" have even been suggested (12) to be the result of the dissolution of intercellular cement. In the hypothesis presented above, the mode of action of ADH has been suggested to be the depolymerisation of the polysaccharide macromolecules in the basement membrane of the descending limb of the loop of Henle.

The following points support the hypothesis suggested in this thesis:

a) If morphological studies are to be taken into consideration no "pores" have been seen in the distal convoluted or collecting tubules in the dehydration or pitressin experiments. On the other hand, a very significant morphological change was noticed in the basement membrane of the descending limb of the loop of Henle in water diuresis.

b) According to the current hypothesis the same amount of fluid runs along both limbs of the loop of Henle until it reaches the distal convolution in both the hydrated and the dehydrated animals, while in the hypothesis suggested here, a large volume of water leaves the descending limb of the loop of Henle in the dehydrated state only, and remains in the loop if the animal was hydrated. In this electron microscopic study, the lumen of the descending limb of the loop was found very narrow in the dehydration experiments and very wide in the hydrated animals.

c) Gottschalk and Mylle (18) by puncturing the loops of Henle and vasa recta of rats with diabetes insipidus have found that the fluid there has an osmolality of about 500 mOsm./Kg water. These same two investigators and others (19) have found the osmolality in the loops of Henle and vasa

recta blood in the concentrating kidney to be about 2200 mOsm/Kg water (it differs in different animals). This is a very strong evidence that an important defect in the functional performance of the loop occurs in absence of ADH. If the loop was acting as a countercurrent multiplier all the time and ADH acted on the collecting tubules, the osmolality of the fluid at the tip of the loop should be about 2200 mOsm/Kg. water, whether ADH was present or absent. On the other hand, if the loop acted as a countercurrent multiplier in presence of ADH only, as suggested in the given hypothesis, the osmolality at the tip of the papilla should be just above isotonicity in absence of ADH, which is exactly what has been found in the diabetes insipidus rats.

d) Ullrich and Jarausch (53) have shown that during water diuresis sodium and chloride are concentrated slightly, in the papilla, urea very little and creatinine not at all. This indicates that during water diuresis no water diffuses out of the descending limb of the loop of Henle into the interstitium and so creatinine is not concentrated at all. The system does not work as a countercurrent multiplier and only slight increase in the concentration of sodium and chloride is due to the initial effect of active sodium transport by the ascending limb into the interstitium, an effect which is not multiplied in water diuresis.

e) The evidence presented above (2,9) of the production of urine of very slight hypertonicity in the region of 500 - 500 mOsm/Kg when the urine flow in the collecting ducts had been slowed enough to effect complete equilibration with the medullary interstitium, in the absence of ADH, is another important evidence for the suggested modified hypothesis.

The biochemical mechanism of the increase in permeability of the renal

tubule produced by the antidiuretic hormone has been recently clarified. Schwartz and his co-workers (43), using tritium-labelled arginine vasopressin, have shown that the binding of the hormone to receptor sites in the mammalian renal tubule and in the toad bladder involves a disulphide-sulphhydryl interchange reaction. They postulate that this reaction produces conformational changes in the proteins of the membrane barrier permitting the more rapid passage of molecules of water. If the type of glycoprotein in the basement membrane of the descending limb of Henle's loop is different from that present in other parts of the nephron as suggested, and if it alone contained the necessary chemical requisites to interact with the disulphide bridge of the antidiuretic neurohypophyseal peptide, it can be explained why this hormone acts only on the basement membrane of this segment of the nephron.

Although the primary action of vasopressin is to increase the permeability of the renal tubule to water, it has been shown also that it increases the permeability to urea and increases the active transport of sodium (30). In the dehydration and pitressin experiments, the cells of the thin segment of the loop of Henle look very active: the basal and luminal surfaces were thrown into numerous complex folds and papilliform processes from these cells were seen to project into the lumen. The increased activity of these cells might be the means by which active Na^+ transport is increased under the influence of ADH. This would further increase the concentration of sodium and the osmolality of the medullary interstitium in these conditions.

It has been recently demonstrated that urea is reabsorbed from the proximal and distal convolutions and collecting ducts but is added to the

tubular fluid in the descending limb of the loop of Henle (29). This was considered to be responsible for the increase in the concentration of urea in the papilla. The thick,impermeable basement membrane of the descending limb of the loop in water diuresis will prevent the diffusion of urea into the thin descending limb and will result into a low concentration of urea at the loop bend in the renal papilla.

Although many of the findings arrived at by this electron microscopic study could fit very properly in the "jig-saw" puzzle of the mechanisms of urinary concentration and dilution, and could explain many of several unresolved obscurities in the countercurrent hypothesis, several other questions have been raised which are difficult to answer at this stage. Why to the collecting tubule cells in the inner medulla separate from each other after forcible hydration? It has been shown that many factors influence cell adhesiveness, and that the degree of hydration of the intercellular cement is one important factor (38). Does the very dilute urine passing along the collecting tubules in marked water diuresis dissolve the intercellular cement and lead to loss of cell adhesiveness?

Another question is the significance of the mitochondrial changes and the vacuoles seen in the light cells of the collecting tubules in water diuresis. Does this indicate an endeavour by these cells to reabsorb Na^+ from the outgoing urine beyond their normal capacity or does it indicate the unphysiologically acceptable alternative of active water excretion? Whatever the answer will prove to be, the close association of the vacuoles to the mitochondria, the disappearance of the mitochondria and their replacement by dense structureless bodies and the pathway of the vacuole between the basement membrane and basal cell membrane has been established.

The experiment of dehydration after forcible hydration has shown an important finding. The changes which occur in the nephron after a physiological dose of water are completely reversible on subsequent dehydration. On the other hand, the changes that follow an unusually large water load, are incompletely reversible. This can beautifully explain the clinical observation that prolonged and excessive drinking in primary polydipsia as well as in diabetes insipidus will gradually diminish the sensitivity of the kidney to the antidiuretic hormone and will eventually lead to a permanent loss of the power of the kidney to conserve water (1, 22).

The study of the mechanisms for the regulation of water metabolism should not seem a far cry from clinical medicine. In the polyuric and polydypsic patient a definitive diagnosis and differential diagnosis is essential to correct treatment; and only correct treatment will restore physiological (and social) freedom to the patient by permitting the elaboration of a hypertonic urine - even as in the bird and in other terrestial mammals.

REFERENCES.

1. Barlow, E.D. and de Wardener, H.E.
Compulsive water drinking.
Quart. J. Med., 28: 235, 1959.

2. Berliner, R.W. and Davidson, D.G.
Production of hypertonic urine in the absence of pituitary antidiuretic hormone.
J. Clin. Invest., 36: 1416, 1957.

3. Berliner, R.W., Levinsky, N.G., Davidson, D.G. and Eden, M.
Dilution and concentration of the urine and the action of antidiuretic hormone.
Amer. J. Med., 24: 730, 1958.

4. Bray, G.A.
Freezing point depression of rat kidney slices during water diuresis and antidiuresis.
Amer. J. Physiol., 199: 915, 1960.

5. Brodsky, W.A., Rehm, W.S., Dennis, W.H. and Miller, D.G.
Thermodynamic analysis of the intracellular osmotic gradient hypothesis of active water transport.
Science, 121: 302, 1955.

6. Burgess, W.W., Harvey, A.M. and Marshall, E.K. Jr.,
The site of the antidiuretic action of pituitary extract.
J. Pharmacol. Exper. Therap., 49: 237, 1933.

7. Crane, M.M.
Observations on the function of the frog's kidney.
Amer. J. Physiol., 81: 232, 1927.

8. Davies, R.E.
The mechanism of hydrochloric acid production by the stomach.
Biol. Rev., 26: 87, 1951.

9. Del Greco, F. and de Wardener, H.E.
The effect on urine osmolality of a transient reduction in glomerular filtration rate and solute output during a 'water' diuresis.
J. Physiol., 131: 307, 1956.

10. Fessler, J.H.
A structural function of mucopolysaccharides in connective tissue.
Biochem. J., 76; 124, 1960.

11. Gersh, I. and Catchpole, H.R.
The organisation of ground substance and basement membrane and its significance in tissue injury.
Amer. J. Anat., 85: 457, 1949.

12. Ginetsinskii, A.G.
Role of hyaluronidase in the reabsorption of water in renal tubules. The mechanism of action of the antidiuretic hormone.
Nature, 182: 1218, 1958.

13. Ginetsinskii, A.G.
Evolution of the water eliminating function of the kidney.
Fiziol. zh. SSSR, 45: 761, 1959.

14. Ginetsinskii, G.A.
The role of system hyaluronic acid-hyaluronidase in the process of water reabsorption in the renal tubules.
Doklady Akademii Nauk SSSR, 119: 1043, 1958.

15. Ginetsinskii, G.A., Sax, M.G. and Titova, L.K.
The mechanism of the action of the antidiuretic hormone.
Doklady Akademii Nauk SSSR, 120: 216, 1958.

16. Glimstedt, G.
Quantitatif hystotopochemische Untersuchungen über die Nieren.
Ztschr. Mikroskop. anat. Forsch., 52: 335, 1942.

17. Gottschalk, C.W. and Mylle, M.
Micropuncture study of the mammalian urinary concentrating mechanism: Evidence for the countercurrent hypothesis.
Amer. J. Physiol., 196: 927, 1959.

18. Gottschalk, C.W. and Mylle, M.
Premier Congres International de Nephrologie.
Evian, 1960.

19. Gottschalk, C.W., Lassiter, W.E., Mylle, M., Ullrich, K.J., Schmidt-Nielsen, B., Pehling, G. and O'Dell, R.
Micropuncture study of the composition of fluid from loops of Henle and collecting ducts in the rodent papilla.
Excerpta medica, 29: 43, 1960.

20. Hargitay, B. and Kuhn, W.
Das multiplikations - principals grundlage der harnkonzentrierung in der Niere.
Z. Electrochem., 55: 539, 1951.

21. Hilger, H.H., Klumper, J.D. and Ullrich, K.J.
Wasserruckresorption und Ionentransport durch die Sammelfohrzellen der Saugetierniere.
Pflugers Arch. ges. Physiol., 267: 218, 1958.

22. Kleeman, C.R., Maxwell, M.H. and Witlin, S.
Functional isosthenuria. An isolated reversible renal tubular defect.
Arch. Intern. Med., 101: 1023, 1958.

23. Klümper, J.D., Ullrich, K.J. and Hilger, H.H.
Das Verhalten des Harnstoffs in den Sammelrohren der Saugetierniere.
Pflügers Arch. ges. Physiol., 267; 238, 1958.

24. Koefoed-Johnsen, V. and Ussing, H.H.
The contributions of diffusion and flow to the passage of D_2O through living membranes.
Acta physiol. Scandinav., 28: 60, 1953.

25. Krakusin, J.S. and Jennings, R.B.
Radioautographic localisation of Na^{22} in the rat kidney.
Arch. Path., 59: 471, 1955.

26. Kuhn, W. and Ryffel, K.
Herstellung Konzentrierter Lösungen aus verdünntendurch blosse Membranwirkung. Ein Modellversuch zur Funktion der Niere.
Ztschr. Physiol. Chem., Hoppe-Seyler's, 276: 145, 1942.

27. Lapp, L.
Elektronmikroskopische Untersuchungen zur Funktion des Nierenmarkes der Ratte.
Verhand. Deutsch. Gesellsch. fur Patholog., 44: 294, 1960.

28. Lassen, N.A., Longley, J.B. and Libienfield, L.S.
Concentration of albumin in renal papilla.
Science, 128: 720, 1958.

29. Lassiter, W.E., Gottschalk, C.W. and Mylle, M.
Micropuncture study of net transtubular movement of water and urea in nondiuretic mammalian kidney.
Amer. J. Physiol., 200: 1139, 1961.

30. Leaf, A.
Some actions of neurohypophyseal hormones on a living membrane.
J. Gen. Physiol., 43: 175, Suppl. Part 2, May 1960.

31. Levinsky, N.G. and Berliner, R.W.
The role of urea in the urine concentrating mechanism.
J. Clin. Invest., 38: 741, 1959.

32. Levinsky, N.G. Davidson, D.G. and Berliner, R.W.
Effects of reduced glomerular filtration on urine concentration in the presence of antidiuretic hormone.
J. clin. Invest., 38: 730, 1959.

33. Lilienfield, L.S., Rose, J.C. and Lassen, N.A.
Diverse distribution of red cells and albumin in the dog kidney.
Circulat. Res., 6: 810, 1958.

34. Ljungberg, E.
On the reabsorption of chlorides in the kidney of the rabbit.
Acta med. Scandinav. Suppl. 186: 1, 1947.

35. Marnery, J.F.
Minerals in nonosseous connective tissue
IN Mineral Metabolism, Vol. I, Part B, 552, 1961.
Editors: Comar, C.L. and Bronner, F.
Academic Press, New York.

36. Peter, K.
Untersuchungen über Bau und Entwickelung der Niere
Jena: Fischer, 1909.

37. Richards, A.N.
Croonian Lecture: Processes of urine formation.
Proc. Roy. Soc. London ser. B. 126: 398, 1938.

38. Rinaldini, L.M.J.
The isolation of living cells from animal tissues.
Inter. Rev. Cytol., 7: 587, 1958.

39. Sawyer, W.H.
The antidiuretic action of neurohypophyseal hormones in Amphibia.
IN The Neurohypophysis, Heller, H, Editor. p. 171-182.
Academic Press Inc., London, 1957.

40. Schmidt-Nielsen, B.
Urea excretion in mammals.
Physiol. Rev., 38: 139, 1958.

41. Schmidt-Nielsen, B. and O'Dell, R.
Structure and concentrating mechanism in the mammalian kidney.
Amer. J. Physiol., 200: 1119, 1961.

42. Schmidt-Nielsen, K., Schmidt-Nielsen, B. and Brokaw, A.
Urea excretion in desert rodents exposed to high protein diets.
J. Cell and Comp. Physiol., 32: 361, 1948.

43. Schwartz, I.L., Rasmussen, H., Schoessler, M.A., Silver, L., and Fong, C.T.O.
Relation of chemical attachment to physiological action of vasopressin.
Proc. Nat. Acad. Sci., 46: 1288, 1960.

44. Smith, H.W.
The excretion of water.
Bull. N.Y. Acad. Med., 23: 177, 1947.

45. Smith, H.W.
The Kidney: Structure and Function in Health and Disease.
Oxford University Press. New York, 1951.

46. Smith, H.W.
Renal excretion of Sodium and water.
Fed. Proc., 11: 701, 1952.

47. Smith, H.W.
Principles of Renal Physiology.
Oxford University Press. New York, 1956.

48. Smith, H.W.
From Fish to Philosopher.
Ciba Pharmaceutical Products Inc. 1959.

49. Smith, H.W.
The fate of sodium and water in the renal tubules.
Bull. N.Y. Acad. Med., 35: 293, 1959.

50. Ullrich, K.J., Drenckhahn, F.O. and Jarausch, K.H.
Untersuchungen zum Problem der Harnkonsentrierung Und verdunnung. Uber das osmotische Verhalten von Nierenzellen und die begleitende Elektrolytanhaufung im Nierengewebe bei verschiedenem Diuresezustanden.
Pflügers Arch. ges. Physiol., 261: 62, 1955.

51. Ullrich, K.J., Eigler, F.W. and Pehling, G.
Sekretion von Wasserstoffionen in den Sammelrohren der Saugetierniere.
Pflugers Arch. ges. Physiol., 267: 491, 1958.

52. Ullrich, K.J., Hilger, H.H. and Klumper, K.J.
Sekretion von Ammoniumionen in den Sammelrohren der Saugetierniere.
Pflügers Arch. ges. Physiol., 267: 244, 1958.

53. Ullrich, K.J. and Jarausch, K.H.
Untersuchungen zum Problem der Harnkonzentrierung und verdünnung.
Über die Verteilung der Elektrolyten (Na, K, Ca, Mg, Cl, anorg. phosphat) Harnstoff, Aminosauren und exogenem Kreatinin in Rinde und Mark der Hundeniere bei verschiedenen Diuresezustanden.
Pflugers Arch. ges. Physiol., 262: 537, 1956.

54. Ullrich, K.J., Kramer, K and Boylan, J.W.
Present knowledge of the countercurrent system in the mammalian kidney.
Prog. Cardiovasc. Dis., 3: 395, 1961.

55. Ullrich, K.J. and Pehling, G.
Activer Natriumtransport und Sauerstoff-verbrauch in der auszeren Markzone der Niere.
Pflugers,Arch. ges. Physiol., 267: 207, 1958.

56. Ullrich, K.J., Pehling, G. and Espinar-Lafuente, M.
Wasser und Electrolytfluss in vascularen gegenstromsystem des Nierenmarks.
Pflugers Arch. ges. Physiol., 273: 562, 1961.

57. Walker, A.M., Bott, P.A., Oliver, J. and MacDowell, M.C.
The collection and analysis of fluid from single nephrons of the mammalian kidney.
Amer. J. Physiol., 134: 580, 1941.

58. Wesson, L.G. Jr., and Anslow, W.P. Jr.
Excretion of sodium and water during osmotic diuresis in the dog.
Amer. J. Physiol., 153: 465, 1 948.

59. Wesson, L.G. Jr., and Anslow, W.P. Jr.
Effect of osmotic and mercurial diuresis on simultaneous water diuresis.
Amer. J. Physiol., 170: 255, 1952.

60. Wesson, L.G. Jr., Anslow, W.P. Jr. and Smith, H.W.
The excretion of strong electrolytes.
Bull. N.Y. Acad. Med., 24: 586, 1948.

61. Winters, R.W. and Davies, R.E.
The role of countercurrent mechanisms in urine concentration: A review.
Ann. Int. Med., 54: 810, 1961.

62. Wirz, H.
Der osmotische Druck des Blutes in der Nierenpapille.
Helvet. physiol. et pharmacol. acta, 11: 20, 1953.

63. Wirz, H.
Der osmotische Druck in den corticalen Tubuli der Rattenniere.
Helvet. physiol. et pharmacol. acta, 14: 353, 1956.

64. Wirz, H.
The location of antidiuretic action in the mammalian kidney
in The Neurohypophysis, Heller, H; Editor. p. 157-169.
Academic Press. Inc., London, 1957.

65. Wirz, H.
Lokalisation des Konzenstrierungsprozecces in der niere durch direkte kryoscopie.
Helv. Physiol. Pharmacol. Acta, 9: 196, 1961.

POTASSIUM DEFICIENCY NEPHROPATHY.

POTASSIUM DEFICIENCY NEPHROPATHY.

INTRODUCTION.

Potassium is the major intracellular cation, and, as such, must play a key role in the inter-relationships between cell structure and function. It is therefore not surprising that a deficit in this mineral is accompanied by alterations in cellular activity and anatomic integrity. The functional disturbances may vary from gross muscle paralysis and abnormalities in the myocardium with disturbed electrocardiographic patterns and conduction defects, to subtle changes in renal tubular activity and disturbances in acid-base balance. The structural lesions consequent on potassium depletion have been observed principally in the kidneys and the heart.

In the renal tubular cells potassium plays a dual role. As in all other cells, it is probably an essential activator of someof the enzymatic reactions maintaining the integrity of the tubular cells and supplying the energy required for its specialised activities. Beyond this, however, potassium is itself one of the ions transported across the renal epithelium as part of the inter-related series of ionic exchanges by which the kidney contributes to the maintenance of body electrolyte and acid-base equilibrium. An increasing body of evidence has been accumulating in the last two decades demonstrating that a deficit of body potassium results in significant alteration in renal structure and function.

In 1919 Jaffe and Steinberg (44) reporting upon a large series of

cases of chronic dysentery which had come to autopsy, noted that 25 per cent showed a specific lesion of the renal tubules. They described it as a "vacuolar degeneration" with ballooning of many of the epithelial cells, particularly those of the proximal convoluted tubules. The vacuoles did not take up stains for either fat or glycogen. The glomeruli and arterioles appeared to be spared. This was the first good description of potassium deficiency nephropathy in man, though the authors did not associate it with a deficiency of that ion. However, that the tubular changes could be"due to disturbed absorption of nutritional substances from the intestinal tract" was one of the possibilities seriously considered.

Clinically, little more was heard about this interesting lesion until 1940 when Ch'in and Hu (10) described it again in autopsy material from cases of bacillary dysentery. In 1947 (94) and again in 1950 (45,50,65, 68) papers appeared associating these tubular lesions with chronic intestinal disease, but only Perkins, Peterson and Riley (68) related the lesion to a deficiency of potassium and were the first to ascribe anatomical changes in the human kidneys to potassium deficiency. This association was soon confirmed by Luft et al (52) in 1951, and since then the relationship of "clear cell nephrosis" or "vacuolar nephropathy" to the state of potassium deficiency has gradually been appreciated.

Knowledge regarding renal pathology in potassium-depleted human beings came entirely from three clinical situations, all of which represent potassium depletion, but in association with other metabolic abnormalities; large and chronic losses of gastrointestinal fluid, primary aldosteronism and renal tubular potassium wasting. The latter group (7,24,52,54,77,99,5 'case 3') does not provide much helpful information about the effects of

potassium depletion on the kidneys since, although many are probably examples of primary aldosteronism (92), there may also be one or another type of primary renal disease.

The prominent histological change described in the kidneys of potassium depleted patients is a vacuolisation of the tubular epithelium, which is localised mainly but not entirely to the proximal convoluted tubule. The vacuoles are usually large and single and contain neither fat nor glycogen. The nuclei of the cells are displaced towards the basement membrane and the cytoplasm is ballooned inwards to project into the tubular lumen. This lesion has now been reported in twenty-three patients; 11 with predominantly gastrointestinal causes of potassium depletion (6,19,33,46,68,73,80,93,94, 98); 11 with primary aldosteronism (2,5a,9,12,13,18,23,26,49,81); and one with gastrointestinal losses but also with chronic glomerulonephritis (7). In 14 of these, the lesions were thought to be mainly proximal (5a,9,18,33, 46,49,68,73,80,93,94,98), the validity of which was established in four instances by microdissection of nephrons (9,18). It is of interest, however, that there are 15 reports in which this 'characteristic' hydropic lesion was apparently absent, 3 with gastrointestinal losses (70,73), 9 with primary aldosteronism (3,5,8,15,57,89,90) and 3 where the potassium wasting was of uncertain cause (5,54,99). This suggests that vacuolisatinn is by no means a constant finding, although the significance of its absence in many of these studies is difficult to evaluate because of varying degrees of potassium repletion prior to obtaining kidney tissue.

While the vacuolar change in the proximal convoluted tubular epithelium is the most frequently reported renal lesion in chronic potassium depletion in man, other less striking alterations in the tubular epithelium have been

described: granularity and dark staining of the cytoplasm (52), foamy swelling of the cytoplasm (50), necrosis and sloughing of cells (12,46,68), dilatation of tubules with atrophy of the epithelium (19,50) and even calcification of the renal parenchyma (12) have been reported. These changes were present either with or without the vacuolar change.

The tubular lesions in the reported human cases seem to be confined chiefly to the convoluted tubules, particularly the proximal; the collecting tubules and the loops of Henle either described as normal or not mentioned at all. In the cases studied by Darmady and Stranack (9,18) where micro-dissection of the nephron was carried out, the collecting tubule was specifically stated to be normal in one instance (9) and was not mentioned in the other three. However, although nephrons were individually dissected, it was almost certainly impossible to study the medullary portion of the collecting system since the material was obtained by renal biopsy. One report only (50) has described additional changes in the distal and collecting tubules of patients who have died in severe potassium deficiency, consisting of a granular degeneration and atrophy of epithelium in these tubular segments.

Glomeruli have also usually been described as normal, although Jensen et al (45) have called attention to the endothelial proliferation in some of their patients with ulcerative colitis, and Siebenmann (80) has beautifully demonstrated vacuolar lesions in the cells lining Bowman's capsule identical with the vacuolar changes in the proximal tubular epithelial cells.

Other structural changes in human kidneys appear to be due primarily to such associated conditions as hypertension in primary aldosteronism (3,5,5a,9,13,15,23,26,57,90), hypertension of uncertain cause (5,99) and

chronic pyelonephritis (5a,8,19,49,52,57,60,70,73,74,93). It is noteworthy that a total of 17 potassium-depleted patients have had complicating pyelonephritis as judged by history, urine cultures or renal histology (2,5a, 8,19,20,49,52,57,60,70,73,74,77,93) although in at least three of these (2,5a,77) the pyelonephritis may well have preceded the potassium depletion. It has been suggested (57) that the potassium depleted kidney is more susceptible to bacterial infection than the normal, and this has been confirmed experimentally in rats (96,97).

In the meanwhile, many experimental studies have been carried out and have demonstrated a pathological renal condition in potassium depleted rats and mice. In 1937 Schrader and co-workers (78) described vascular lesions in the proximal convoluted tubules of rats fed on potassium deficient diet for a long time. This was later confirmed (21,28,87) and a similar lesion was reported in potassium deficient mice (51). Macroscopically the kidneys have been reported to be enlarged by most investigators. It is generally agreed that solids and water are both increased, either without change in their relative proportions or with a slightly greater proportional increase in water content. Fat appears to remain a constant percentage of the total kidney weight. In one study (92) the ratio of desoxynitronucleic acid-phosphorus to fat-free dry solids was the same as that of pair fed controls suggesting that true hyperplasia causes the renal enlargement. The increase in renal mass begins within a few days and progresses for ten days to two weeks, the final weight often being two or more times normal (4,83).

Microscopically, there is considerable diversity in the description of the nature and location of the lesions. Essentially all investigators have agreed on the predominantly tubular location of the lesions and on the

presence of tubular dilatation. With the exception of two studies (21,51) all the investigators till 1954 described the experimental renal lesions of potassium deficiency to be localised only to the convoluted tubules, particularly the proximal (17,28,37,48,56,78,87). On their nature there has been no unanimity of opinion. Fatty (28), vacuolar and hydropic degeneration (17,21,27,28,37,48,78,83) and even a general tubular necrosis which in its severity is compared to the 'necrotising nephroses' of heavy metal poisoning have been noted (28), along with the progressive changes of epithelial hyperplasia (21,28,48,51,83) and cystic (28,48,83) dilatation. In 1954 Spargo (83) reported that though a moderate hydropic change occurs in the proximal convoluted tubules in potassium deficient rats, the maximum changes are noted in the collecting tubules. He described proliferation of the cells of the collecting and distal convoluted tubules as well as a hyaline droplet change in the cells of the collecting tubules. Two groups of investigators only (21,51) have previously reported collecting tubule lesions in kidneys of potassium deficient animals. Since then, most investigators have emphasised the predominance of alterations in the collecting tubules, the proximal tubules either not described at all, or reported to be normal or to be the site of an inconstant vacuolar change (14,32,58,66,86,91). Many recent investigators reported that the nature of these collecting tubule lesions is swelling, hyperplasia and prominent cytoplasmic granulation of the epithelial cells (14,51,66,83,91) as well as enlargement of nuclei (32). Some described only one feature of these collecting tubule changes, e.g. Milne et al (58) described in detail the accumulation of numerous deeply eosinophilic, PAS positive, hyaline, spherical granules in the cells of the distal three-quarters of the collecting

tubules and stressed not only their frequency in the ducts near the papilla but their occurrence in the epithelial covering of the pelvis. They, however, did not report any cellular proliferation. However, even some of the more recent studies report lesions of a different nature as necrosis of tubular cells and vacuolation of their cytoplasm (30,86).

The interpretation and comparison of these numerous results is, however, hampered in several ways (92). With four exceptions none of these studies provide data regarding the degree of potassium depletion (30,51, 66,69). Except where pair feeding with controls has been employed (28,48, 66,83) it is difficult to be sure that some abnormalities did not result from nutritional deficiencies apart from potassium. Also, the exact localisation of the lesions within nephrons that have been pathologically altered is uncertain with the exception of the single study in which microdissection has been employed (66). For these reasons it is not surprising that the reported pathological changes have been rather divergent.

In a well controlled study with pair feeding and chemical data on the degree of potassium deficiency, Oliver and his co-workers (66) have demonstrated by microdissection that the lesions of potassium deficiency in the rat affect all collecting tubules uniformly, and that different lesions occur in separate portions of the collecting system. The granular lesion was found in the innermost zone of the medulla, which was otherwise normal, and extended out to the epithelium of the papilla. Swelling and hyperplasia of the "clear" cells and a distinctive proliferation of the "intercalated" cells of the collecting tubules was confined to the tubules of the inner stripe of the outer zone of the medulla. This latter lesion frequently

caused apparent luminal obstruction and consequent dilatation of the more proximal portions of the collecting system and occasionally extended up as high as the distal convoluted tubule or even the ascending limb of the loop of Henle, but otherwise the loops of Henle and the distal convolutions were considered normal. These workers further noted that except for minor patchy degenerative changes in the mid-portion of the proximal convolution, the remainder of the nephron was essentially normal.

Interstitial tissue changes have rarely been mentioned, but some studies have shown alterations of intertubular ground substance and/or basement membranes in the medulla (14,66,67) as well as apparently swollen interstitial cells which were PAS positive (14,67). Inflammatory cell infiltration was not reported in uncomplicated potassium depletion; neither were glomerular nor vascular changes, with the exception of one study (30) where atrophic hyalinised glomeruli were reported to result from prolonged potassium deficiency.

A variety of enzymatic changes have been reported in the kidneys of potassium depleted rats. Methods have varied and have involved both whole tissue analyses (43,63,83) and histochemistry (14,59,67,83,91). In general, however, there is a paucity of interpretable data on this obviously important problem in that comparatively few enzymes have been studied by more than one technique. Assays for carbonic anhydrase and glutaminase in the kidneys of potassium depleted rats have shown an increase in the activity of both enzymes as compared to pair fed controls (43,63)while arginase and aminoacid oxidase were found unchanged (43). Enzyme activity as determined by histochemical methods showed changes found mainly in the areas that appear to be most affected in ordinary histological sections.

An increase in acid phosphatase (14,67,91), TPN diaphorase (67) and non-specific esterase activity (67,91) and a decrease in DPN diaphorase activity (67) in the collecting tubules in the medulla have been described. The increased acid phosphatase and non-specific esterase activity was reported to be more marked in the thin limbs of Henle's loop and in the interstitial cells in the medulla (91). It has been reported that non-specific esterase activity is increased in the proximal tubules (67) but using somewhat different histochemical methods; other investigators have found it unchanged (14,83). Alkaline phosphatase has been found to be low (59,83) and unchanged (14,67). Succinic dehydrogenase was found normal in two studies (14,67) and was reported to be reduced in the ascending limb of Henle's loop in a third study (91). Adenosine triphosphate, DPN and TPN diaphorase activities were also reported to be reduced in the ascending limbs of Henle's loops and distal convoluted tubules (91), while 5-neucleatidase and phosphorylase were found normal (14,67). In general, however, both the meaning and the validity of these various reported changes remain uncertain.

The rat and mouse are the only experimental animals in which the renal structural pathology of potassium depletion has been described. Twice in potassium depleted dogs it has been looked for with entirely negative results (71,82). This is of considerable interest and deserves further investigations, particularly since impairment of the renal concentrating ability, as occurs in the human and the rat, does result from potassium depletion in the dog (31).

Essentially complete restoration of normal renal architecture occurs within two to three weeks following potassium depletion in rats and mice

(51,58,86), although there may still be some dilatation of tubules and occasional hyalinisation of glomeruli (51,86). Prompt recovery could also be accomplished with rubidium rather than potassium (74); which could also prevent renal structural changes in potassium-depleted rats (29). The granular lesion of collecting tubules in the inner medulla disappears extremely rapidly following potassium repletion (40,58,59,67); the hyperplastic collecting duct lesion takes a much longer time to subside (40). Most, but not all of the enzymes studied by Pearse and Macpherson (67) returned to normal within seven days following potassium repletion. Thus, the concensus of opinion has been that the renal structural abnormalities which develop with potassium depletion are largely reversible. Two studies (30,51) however, are in conflict with thisconclusion, particularly that of Fourman et al (30) where very severe pathological renal condition has been demonstrated as long as seven months after complete potassium repletion. In another comparable study (40) the results were qualitatively similar to those of Fourman et al but far less marked. It is possible that a greater renal damage in the former study was the result of superimposed pyelonephritis, potassium-depleted rats having an increased susceptibility to renal infection (96,97). Relman and Schwartz (73) have provided the only available information regarding reversibility of potassium depletion nephropathy in man. Serial biopsies in two patients with potassium depletion secondary to gastrointestinal losses showed complete repair several months after potassium repletion (except for some "pyelonephritis" scars in one).

The most prominent abnormality in the renal function in experimental potassium depletion is a defect in the concentrating power. This has been

established both in the rat (41,58,75) and dog (31); and in rats the magnitude of this impairment appears to be proportional to the degree of tissue potassium depletion (41). In humans a similar defect in the urinary concentrating mechanism has been the most prominent and consistent abnormality of renal function noted in association with potassium depletion. Thus, there are thirty-three instances of definite potassium deficiency either due to gastrointestinal losses or to primary aldosteronism in which maximum urinary concentration was specifically tested (2,3,5,5a,9,12,13,15, 16,22,23,26,36,38,39,42,49,53,57,70,73,79,81,89,90) and in all but two cases (16,26) concentrating power was clearly impaired. In an additional seven cases with potassium depletion of uncertain causation the maximum achievable urinary concentration was also subnormal (5,24,52,54,77,85,99). In the vast majority of all of these cases the maximum urinary specific gravity was less than 1.015. This case material also demonstrates that, as in experimental animals the concentrating defect is not related to extracellular acid-base status or to high levels of adrenal corticosteroids (92).

Potassium repletion in rats restores normal urinary concentrating ability quickly (40,58) and this is also true, or nearly so, for the dog (31). Among the human cases there were eight examples of complete repairability following potassium repletion (3,23,36,39,42,79); thirteen instances demonstrating partial restoration of urinary concentrating power (12,13,15,22, 38,54,70,73,81,89,90) and only three case reports showing no improvement in urinary concentrating power following potassium repletion (2,9,57).

It has been suggested (66) that the urinary concentrating defect may in some way be the result of the lesions which have been demonstrated in the collecting tubules. This might be a reasonable hypothesis in view of the

current suggestions that final concentration of the urine occurs in the collecting tubules (34,95). However, the loops of Henle and the distal convoluted tubules are also importantly involved in the renal concentrating process (34,95). Also the absence of significant lesions in the collecting tubules in the human material and the dog is noteworthy. The difference may well be due to an histologically inapparent but functionally important lesion in the human collecting tubule. On the other hand, it is possible that potassium depletion impairs a step in the urine concentrating mechanism which precedes the final distal reabsorption of water.

Other defects in the renal tubular functions have also been reported. An inability of the kidney to produce a highly acid urine (11); decreased excretion of organic acids (25); increased urinary ammonia (58), increased tubular reabsorption of bicarbonate and decreased capacity to excrete a load of sodium bicarbonate (25); reduced excretion of phenol red (73); a fall in PAH extraction and excretion (61,73,79); and a fall in the maximal tubule secretory capacity for PAH (^{Tm}PAH) (73); have been reported. The glomerular filtration rate is reduced in some cases of potassium deficiency (9,24,47,53,61,73,74,79). The serum NPN, BUN or creatinine have been elevated in a few patients (1,20,39,73). Potassium depleted patients usually have either mild proteinuria or none, and it is not certain whether this is of glomerular or tubular origin; it may be related to necrosis of tubular cells (58). The urinary sediment is most commonly unremarkable, but occasionally contains a few red cells, white cells and/or casts.

The above review of clinical and experimental nephropathy due to depletion of potassium established a definite functional and pathological paradox. The excretion of a large volume of dilute urine, though it

can be explained by a lesion in the rat's and mice's collecting tubules, cannot be accounted for by an inconstantly present lesion in the human's proximal tubules. Secondly, even in rats and mice, such functional defect has always been noticed to develop very early in the course of potassium depletion, usually within 48 hours or a few days, while most observers found the collecting tubule lesions only after several days or a few weeks. In view of this and of the fact that potassium depletion might impair a step in the urinary concentration which precedes the final reabsorption of water from the collecting tubules, an electron microscopic study of the nephron of the rat suffering from potassium depletion was undertaken. In this study particular attention was directed towards the discovery of abnormalities, in the renal ultrastructure in mild potassium depletion before lesions are visible by light microscopy, and to correlate these abnormalities with the disturbed renal function. It was thought that lesions might be detected by the electron microscope that cannot be resolved by the light microscope but which are nevertheless functionally significant and might therefore remove much of the controversial concepts about potassium deficiency nephropathy.

MATERIAL AND METHODS.

40 adult white rats, Wistar strain, weighing initially 280-400 gm. were divided into the following groups:-

Group A. 10 rats were given a potassium free diet for 2 weeks.

Group B. 7 rats were given a potassium free diet for 4 weeks.

Group C. 7 rats were given a potassium free diet for 6 weeks.

Group D. 8 rats were given a potassium free diet for 6 weeks but KCl was

added to the drinking water in a concentration of 22 m.equiv./l.

Group E. 8 rats were given a potassium free diet for 6 weeks to which KCl has been added in the concentration of 1 gm/Kgm. of diet. KCl was also added to the drinking water in a concentration of 40 m. equiv./l.

The potassium free diet was that described by Manitius and co-workers (55) with the modification of replacing KI in their formula with NaI.

Low Potassium Diet for Rats.

Basic Diet	add 1000 g.
NaCl	6.04 g.
$NaH_2PO_4H_2O$	4.09 g.
Mineral mixture	30.37 g.
Basic Diet	
Sucrose	458 g.
Lard	220 g.
Vitaminised casein	250 g.
Vitaminised corn oil	50 g.
Choline chloride	4 g.
Mineral Mixture	
$CaCO_3$	2400 g.
$CaHPO_4$	496 g.
$MgSO_4$	1186 g.
$MnSO_4$	30 g.
$ZnCl_2$	2.0 g.
$CuSO_4$	2.4 g.
NaI	6.4 g.
$CoCl_2$	2.4 g.
Fenric citrate	220 g.
Such diet contains	
Na	133 m.equiv./Kg.
Cl	103 m.equiv./Kg.

The potassium concentration of the skeletal muscles was estimated in 4 animals from each group. These animals were exanguinated through the abdominal aorta under light amytal anaesthesia. The blood was heparinised and the plasma potassium and chloride concentrations were estimated by the

use of an EEL flame photometer and poteniometric titration respectively.

Samples of skeletal muscle were dissected free of obvious fat and connective tissue, cut in small pieces and extracted in hot water as described by Relman et al (72). Total muscle water was found by drying about 4 grams of muscle to constant weight. Intracellular potassium concentration was then expressed in terms of "intracellular water" which was taken to equal the difference between total water and the chloride space.

The other rats in each group were put in individual metabolism cages and the urine and faeces were collected daily for potassium-balance studies.

The right kidney was prepared for electron microscopy and the left kidney for light microscopy. In taking blocks for electron microscopy, particular care was made to choose blocks from the cortex, the outer zone and the inner zone of the medulla. Blocks were fixed in 1% buffered osmium tetroxide for one hour, embedded in methacrylate and araldite and examined in an A.E.I.-6 Electron Microscope, as previously described.

RESULTS.

In the potassium deficient rats, Groups A, B and C, three phases of polyuria were noticed.

1) Primary phase: commencing from the 2nd to the 6th day of starting the potassium deficient diet. The urine volume increased from two to five folds. These were observed in all the rats.

2) Secondary phase: from the 12th to the 16th day, where the urine volume increased about threefold. This was noticed in half the rats only.

3) Tertiary phase: from the 20th to the 26th day where the urine volume increased about two to threefold. This was observed in half the rats only.

As reported by others (41,55,62), the potassium-depleted rats lost weight. On average, they last after two weeks 3% of their body weight, after 4 weeks 23% and after 6 weeks 35%. It is interesting that rats in Group D also lost some weight after 6 weeks on the partially supplemented diet. They lost an average of 9% of their body weight, while the fully supplemented rats in Group E gained an average of 5%.

The results of potassium balance studies and of muscle potassium are seen in Tables 9 and 10. These show that the animals on the potassium free diet lost about 20% of their body potassium after 6 weeks and that their muscles became depleted to the extent of between 12 to 16% of the control groups and that the serum potassium was reduced below normal value after 6 weeks of the potassium free diet.

Table 9.

Duration of the K-depleted diet	Rat No.	-ve K-balance(m.Equiv/Kg. body weight)
Two weeks	1	2.5
	2	2.4
	3	1.5
Four weeks	1	1.8
	2	2.5
	3	2.4
Six weeks	1	4.7
	2	3.5
	3	5.1

Table 10.

Serum and Muscle potassium in the control and potassium deficient rats.

Diet & Duration	Rat No.	Serum Potassium m.equiv./l.	Serum Chloride m.equiv./l.	Intracellular Potassium m.equiv/l intracellular water	
Group E	4	4.1	100.2	136	Average
"control"	5	4.7	100.0	146	
6 weeks	6	5.0	100.2	138	142
	7	4.6	100.0	147	
Group D	4	4.7	91.0	143	
Partially	5	4.9	93.7	136	
suppl.6	6	4.0	97.1	124	133
weeks	7	4.3	96.7	129	
Group A	4	3.6	99.3	129	
Low K-	5	4.8	97.3	128	
diet	6	3.4	96.3	101	122
2 weeks	7	4.8	93.8	131	
Group B	4	4.6	97.7	125	
Low K-	5	4.3	97.2	129	
diet	6	-	88.2	132	125
4 weeks	7	3.6	100.8	126	
Group C	4	2.9	86	120	
Low K-	5	2.9	95	114	
diet	6	-	93	121	119
6weeks	7	-	93	120	

Light Microscopy.

In the potassium-deficient animals, light microscopy in general revealed no gross abnormality; in the six weeks deficient rats, however, there was a slight proliferation of collecting tubule cells with some excessive granularity of the cell cytoplasm. This was quite obvious in one animal but minimal in the others. Rats deficient for two and four weeks showed no significant departure from the normal appearance found in the control animals.

Electron Microscopy.

After two weeks potassium depletion (Group A)

1) The most obvious lesion was in the basement membrane of the thin segment of the loop of Henle. The basement membrane was much thickened (between 3000 - 5000 Å "Normal 1000 Å) and looked fibrillar. (Fig. 227). The thickening in some areas was very marked and looked like a meshwork. This lesion was constantly observed in the thin segments in all the rats. It affected all the thin segments in the outer medulla and most of those in the inner medullary zone. The lesion was very marked after two weeks and did not show further progression after four or six weeks.

2) Thickening and fibrillation of the basement membrane of the pars recta of the proximal tubule. This was observed in most tubules in all the rats.

3) In only three rats, swelling and vacuolisation of the mitochondria of some proximal tubule cells were noted (Fig. 228). This has not been noted in the other rats depleted for two weeks (Fig. 229) or in any of the rats depleted for four or six weeks. This lesion is most probably not directly related to potassium depletion. It has never been seen in araldite embedded material. The fact that it looks like the artefact produced by faulty polymerisation of methacrylate makes me think that it is most probably an artefact.

After four weeks potassium depletion(Group B)

1) The following lesions were observed. Thickening and fibrillation of the basement membrane of the thin segment of the loop of Henle (Fig. 230)

2) Thickening and fibrillation of the basement membrane of the pars recta of the loop of Henle (Fig. 231).

3) Swelling, vacuolisation and the appearance of dense osmiophilic bodies within the mitochondria of a few light cells in the collecting tubules in the inner medullary zone (Fig. 232).

After six weeks potassium depletion (Group C)

1) The same thickening and fibrillation already observed in the basement membrane of the thin segment and pars recta of the proximal tubule (Fig. 233,234).

2) Definite lesions in the collecting tubules which differed according to the location of the collecting tubule. These lesions affected only some and not all the collecting tubules,

a) In the cortex: Some collecting tubules were dilated. The lining cells of these dilated tubules appeared normal.

b) In the outer zone of the medulla: Some collecting tubules showed proliferation of their lining cells, particularly the dark cells, with encroachment on or even complete obstruction of the lumen. (Fig. 235). Some of these hyperplastic cells were degenerate. Two types of degeneration were observed.

i) The cell shrinks, its cytoplasm becomes densely osmiophilic, its microvilli approximated and its mitochond ia closely packed together. The cytoplasm becomes vacuolated and the cell becomes eventually necrotic (Fig. 236). This type of degeneration probably characterises the dark cells.

ii) The cell becomes swollen, its cytoplasm rarified and its processes withdrawn from their connections with neighbouring cells (Fig. 237). The mitochondria change into dark, dense, structureless bodies, and the cell is eventually extruded and shed off into the tubular lumen.

c) In the inner zone of the medulla: The collecting tubules were neither dilated nor did they show any degree of cellular proliferation.

However, the following abnormalities were noticed:

i) In many cells the cytoplasm was full of many dark, densely osmiophilic structureless granules (Fig. 238). These granules have been demonstrated to develop within the cytoplasmic mitochondria. The mitochondrion swells, becomes vacuolated and a dense structureless body appears within it, (Fig. 239,240) and eventually replaces it. Though many transitory and intermediate forms of this process have been demonstrated, most of the cells were actually full of these dense granules. Some collecting tubules were still, however, lined by entirely normal cells.

ii) Degenerative changes were frequent among these granulated cells, similar to type ii) described above in the outer medullary zone, (fig. 241, 242). Some tubules appeared to have all their lining cells completely degenerate (Fig.243).

iii) Lateral separation between adjacent cells beginning apparently on the basement membrane side of the tubule, the last point to give way being the terminal bar, (Fig. 244,245).

3) The capillaries in the inner zone of the medulla showed an advanced l esion in their endothelial lining after six weeks potassium depletion. Normally, these inner medullary capillaries are lined by an attenuated fenestrated layer of cytoplasm with slightly thicker areas of cytoplasm distributed in a patchy manner over the capillary basement membrane (Vide Supra) The cytoplasm hardly contains cell organelles. After six weeks depletion of potassium, practically all the inner medullary capillaries showed an immense swelling of their lining endothelial cytoplasm which became very rich in mitochondria, that are already degenerate, swollen and vacuolated, (Fig. 246, 247) with dense structureless bodies appearing in them (Fig. 248).

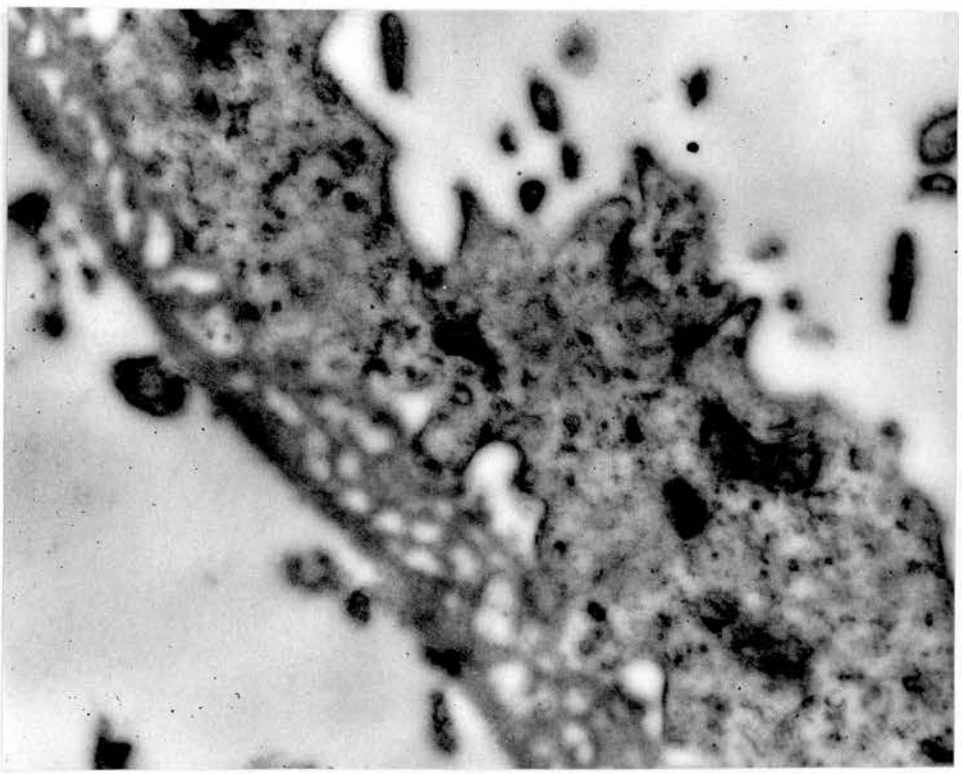


Fig. 227. Thin segment of a loop of Henle in the inner medulla of a two-week potassium deficient rat. Note the thickness and the appearance of the basement membrane.
x 24,000

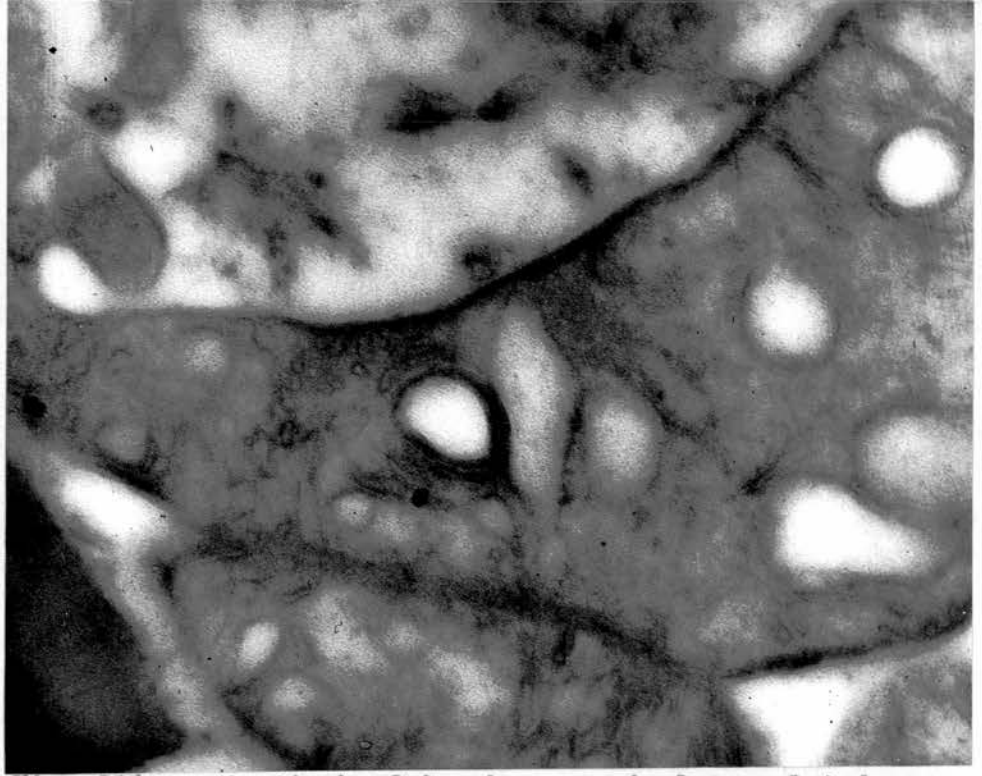


Fig. 228. A mitochondrion in a proximal convoluted tubule cell of a two-week potassium deficient rat. The mitochondrion is swollen, vacuolated and its internal structure is abnormal. Compare with Fig. 229. x 60,000

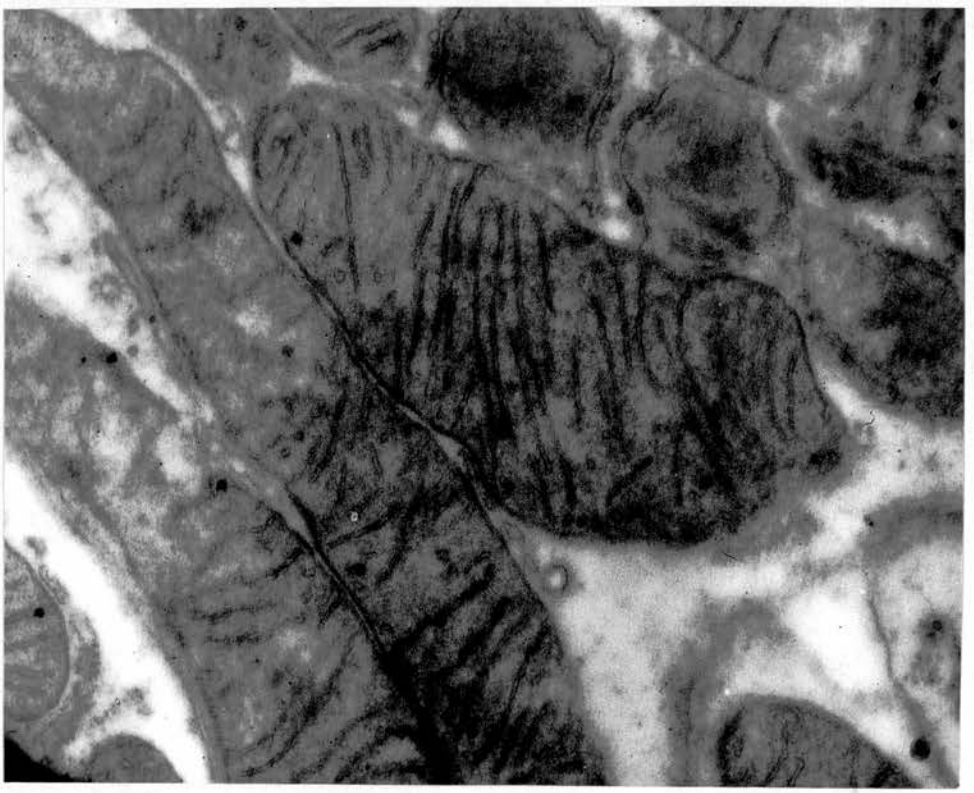


Fig. 229. Proximal convoluted tubule mitochondria from a two-week potassium deficient rat, looking absolutely normal. x 45,000

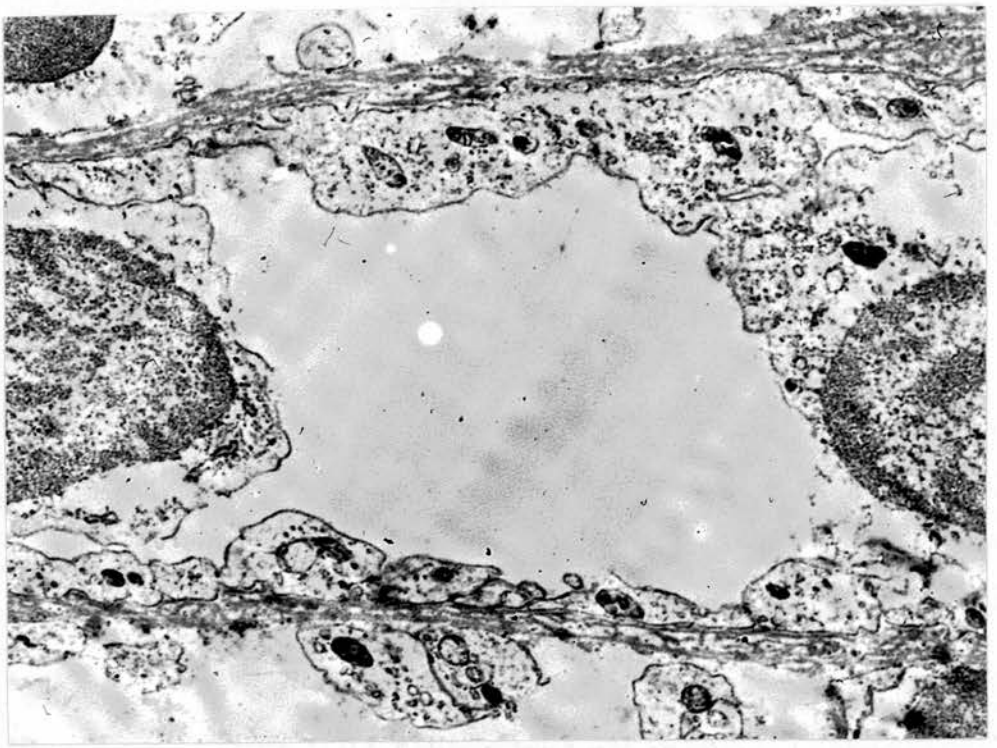


Fig. 230. An outer medullary thin segment from a four-week potassium deficient rat. Note the thickness and fibrillar appearance of the basement membrane. x 9,000

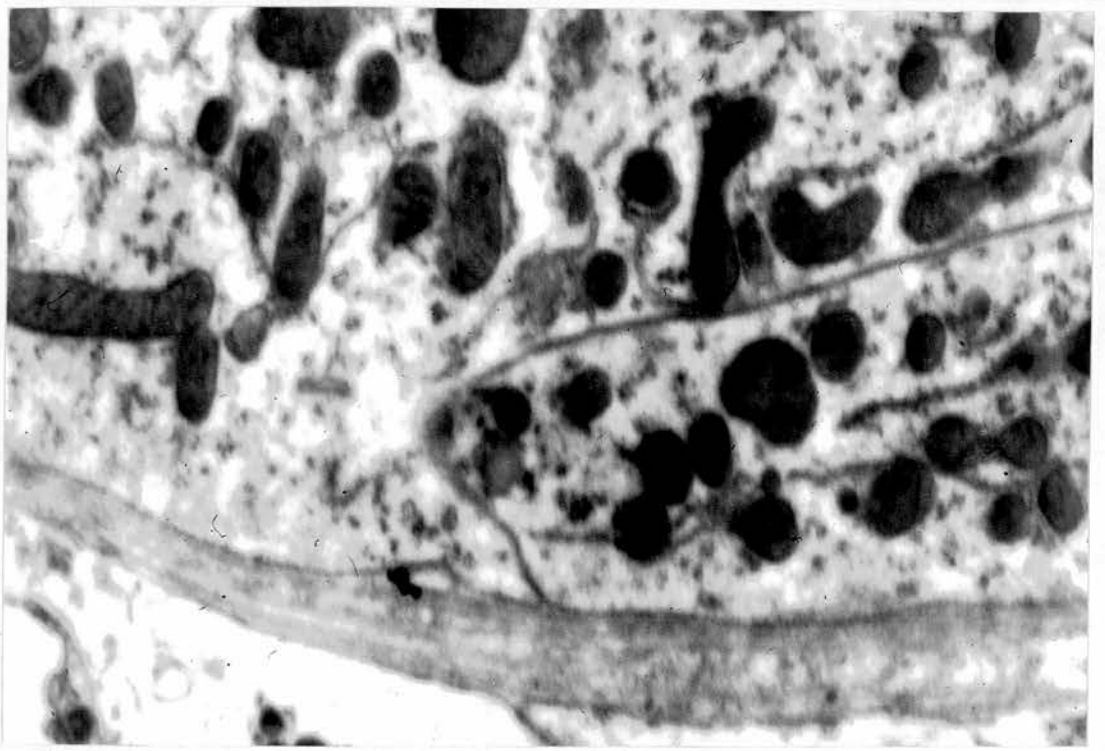


Fig. 231. The basement membrane of a pars recta of the proximal tubule, from a four-week potassium deficient rat much thickened and fibrillar. x 24,000

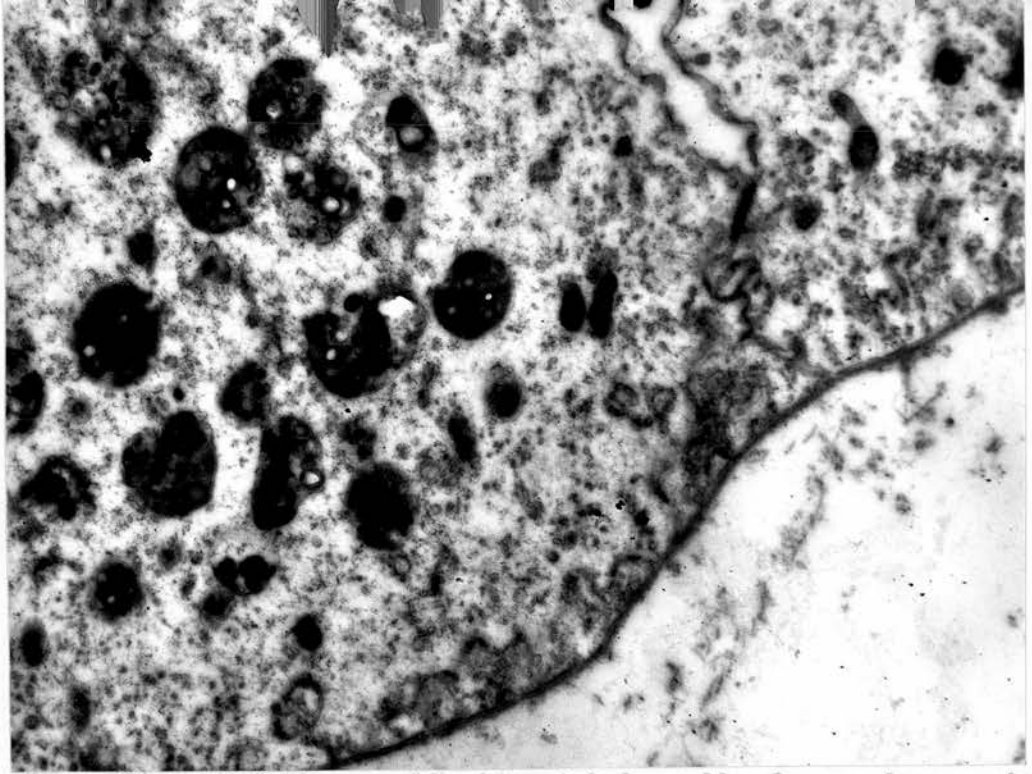


Fig. 232. Medullary collecting tubule cells from a four-week potassium deficient rat. The mitochondria are vacuolated and contain dense osmiophilic bodies. x 15,000

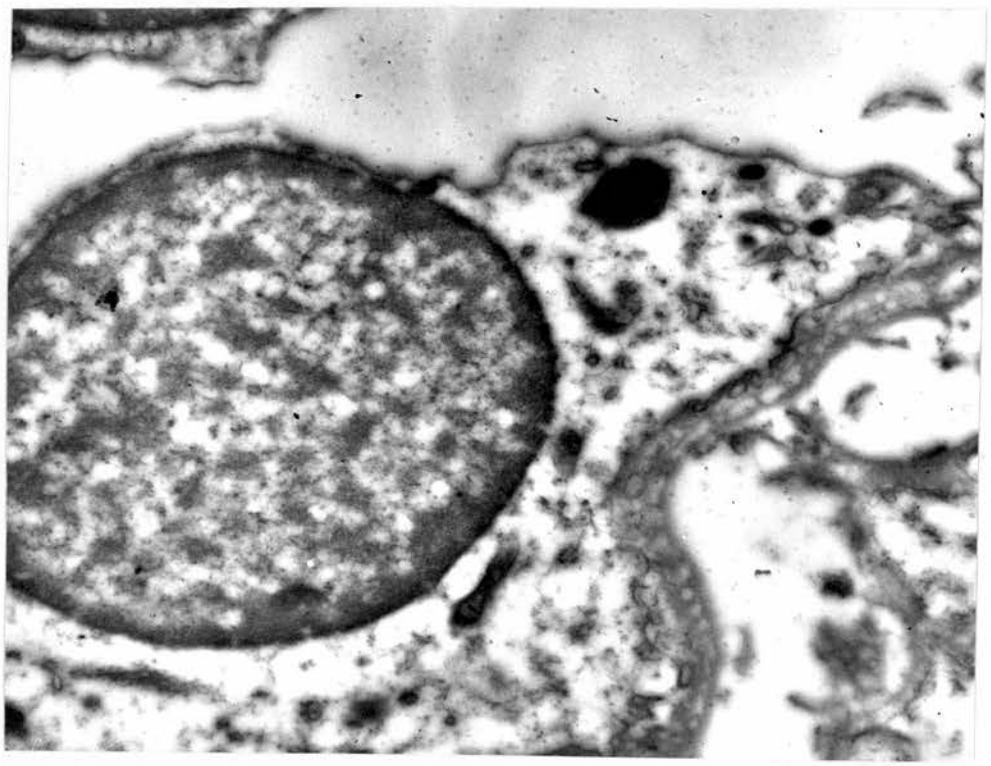


Fig. 233. An outer medullary thin segment from a six-week potassium deficient rat. Note the thickness and appearance of the basement membrane. x 12,000

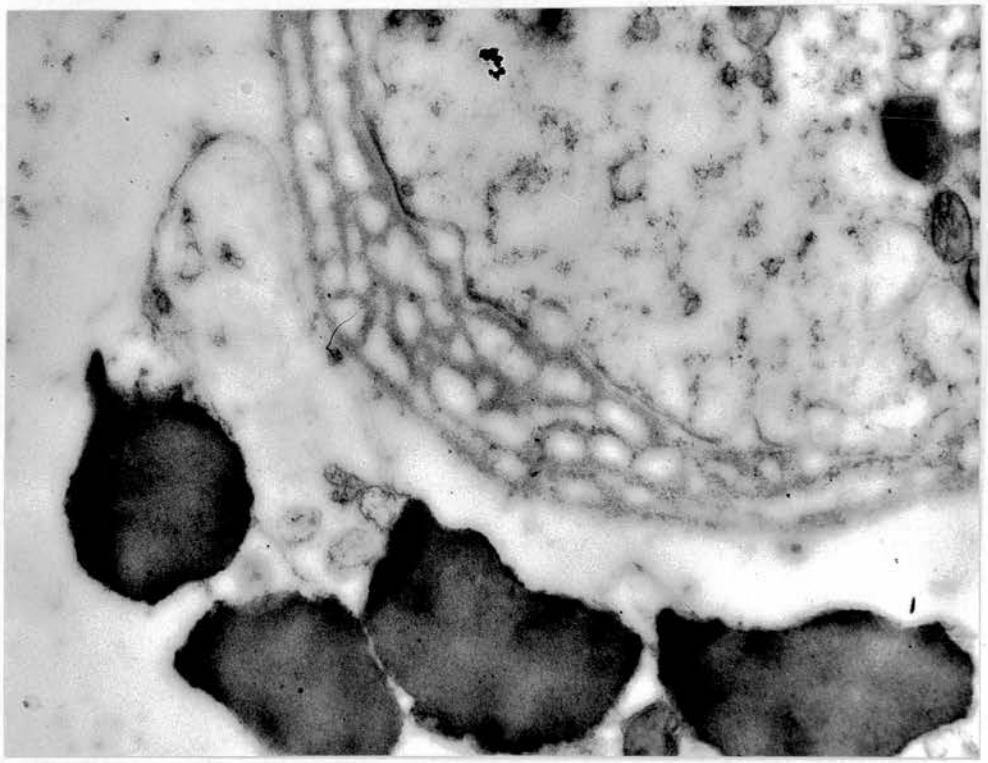


Fig. 234. The basement membrane of a thin segment in the inner medulla of a six week potassium deficient rat. It is markedly thickened and fibrillar and it looks like a meshwork. x 24,000

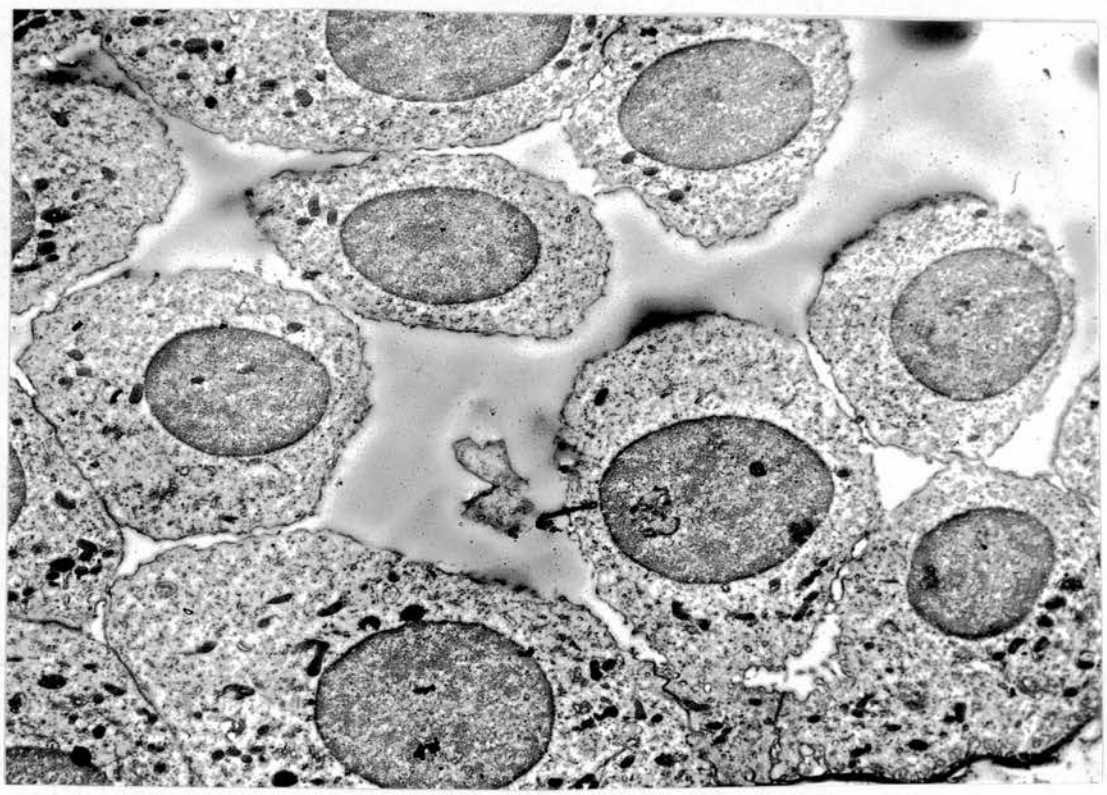


Fig. 235. An outer medullary collecting tubule in a six-week potassium deficient rat. Note the proliferating cells nearly occluding the lumen. x 2,500

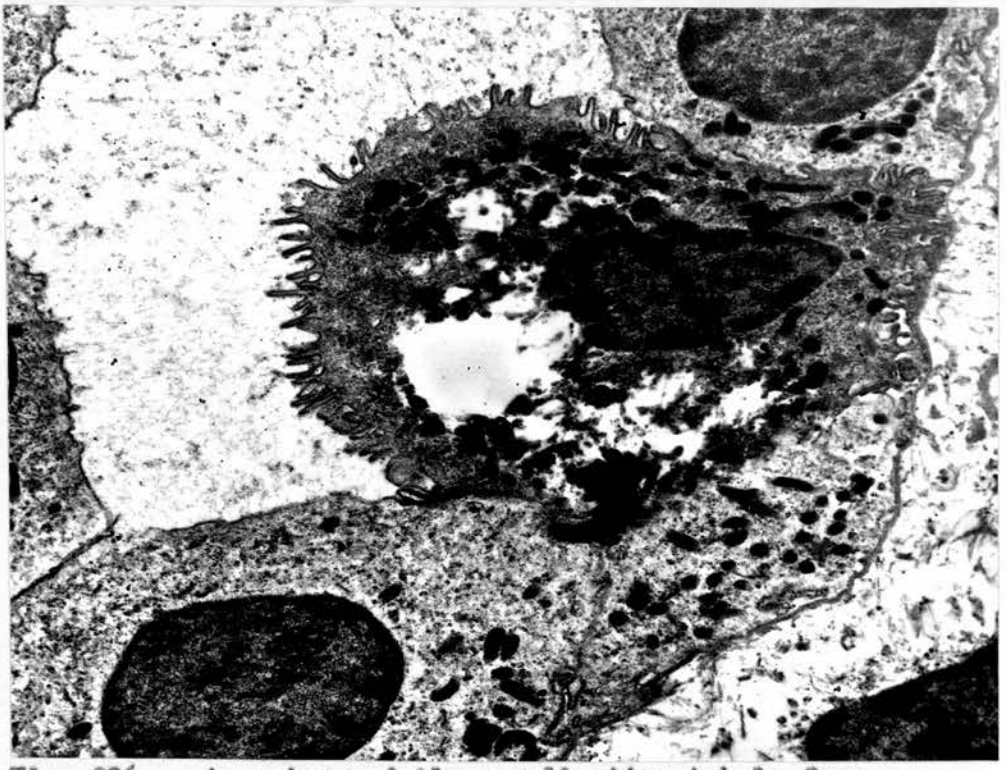


Fig. 236. An outer medullary collecting tubule from a six week potassium deficient rat. Note the shrunken degenerate cell with the crowded mitochondria in the dark cytoplasm and the approximated microvilli. x 4,000

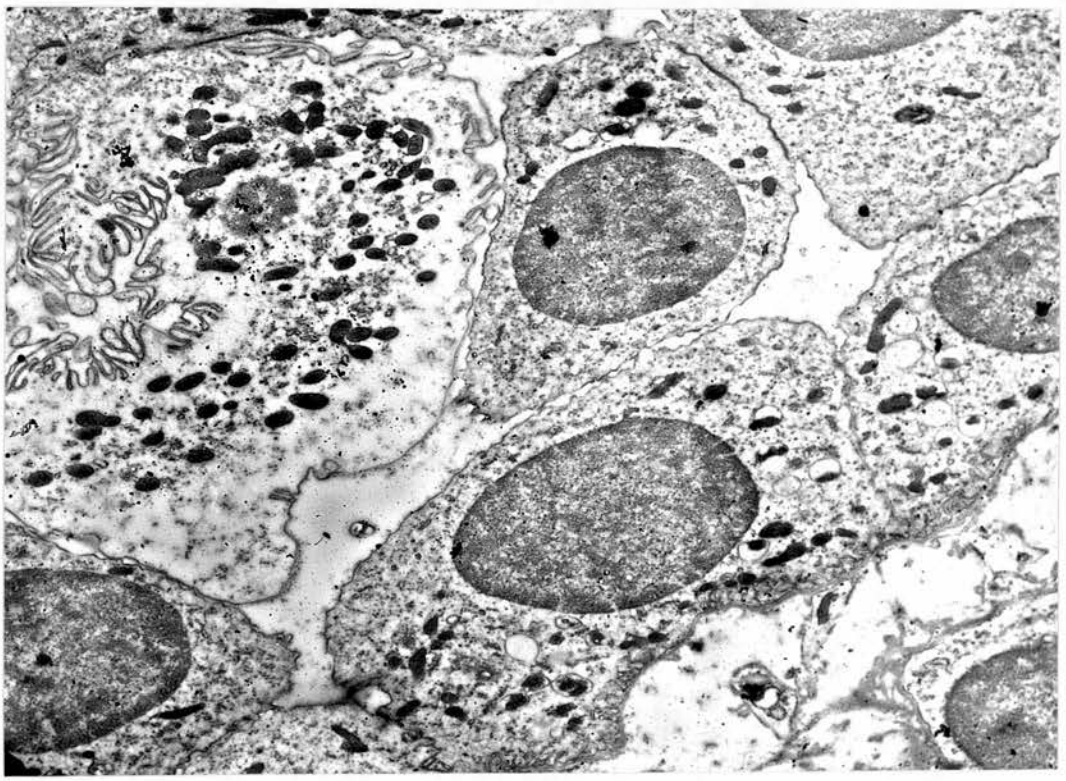


Fig. 237. An outer medullary collecting tubule from a six week potassium deficient rat. Note the hyperplastic cells, and the granulated vacuoles in the cytoplasm. On the left a degenerate cell is seen, swollen, its cytoplasm rarified and its processes withdrawn from adjacent cells as it is being shed off into the lumen. x 4,000

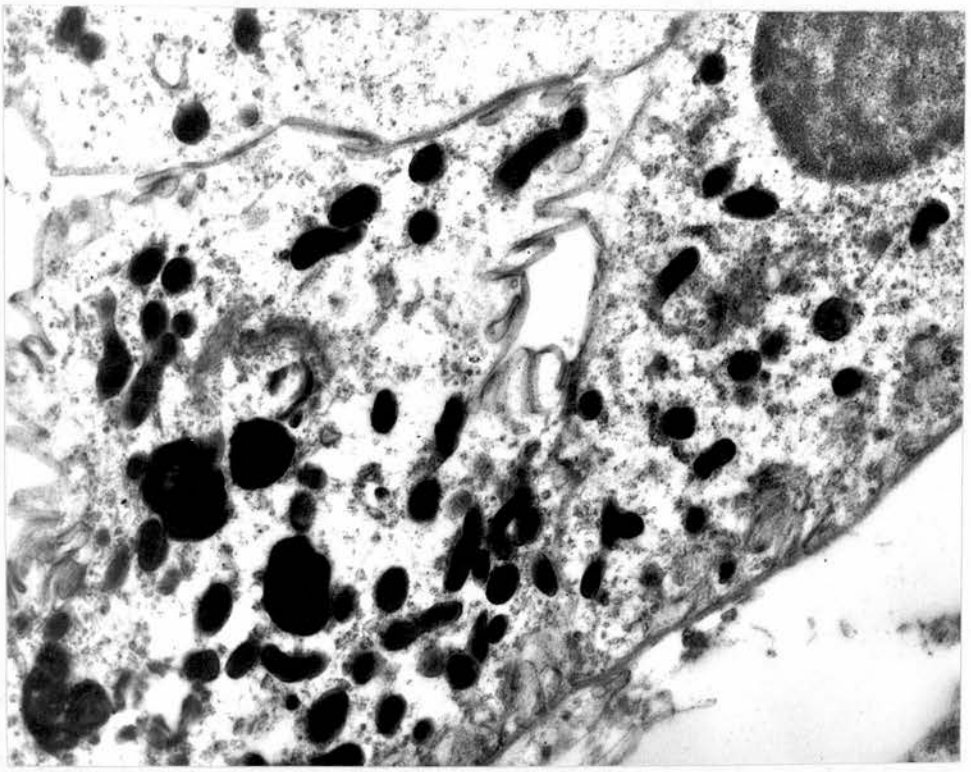


Fig. 238. An inner medullary collecting tubule from a six week potassium deficient rat. The light cells are seen to contain numerous densely osmiophilic granules. x 9,000

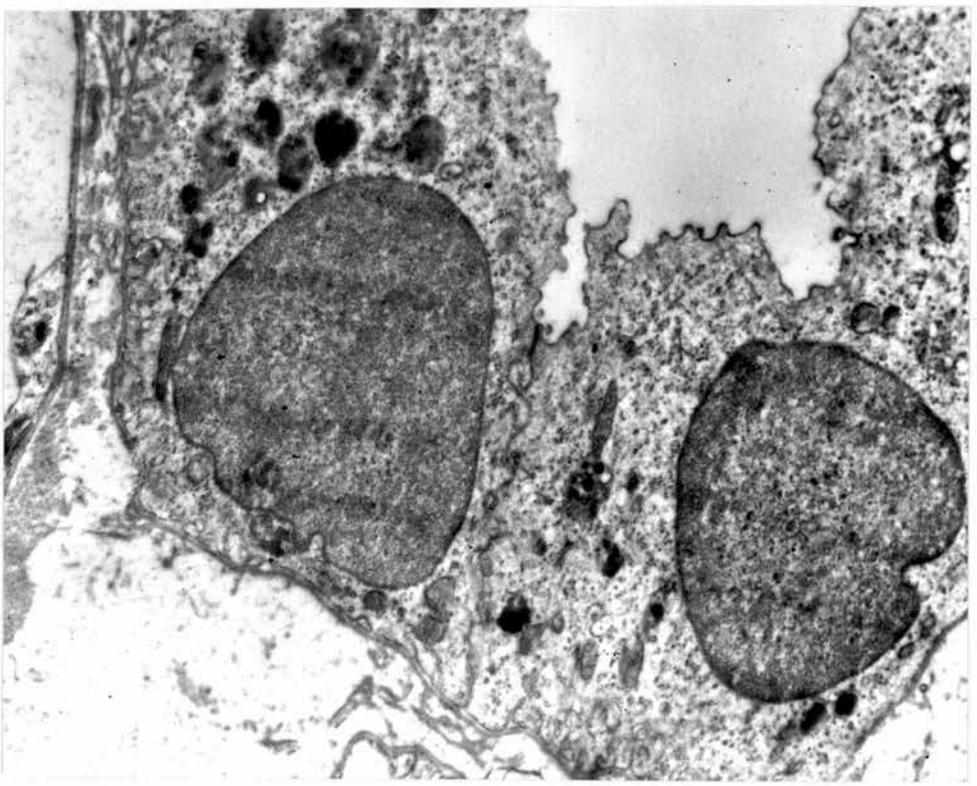


Fig. 239. An inner medullary collecting tubule from a six week potassium deficient rat. Note the vacuoles and the dense bodies appearing within the mitochondria. x 9,000

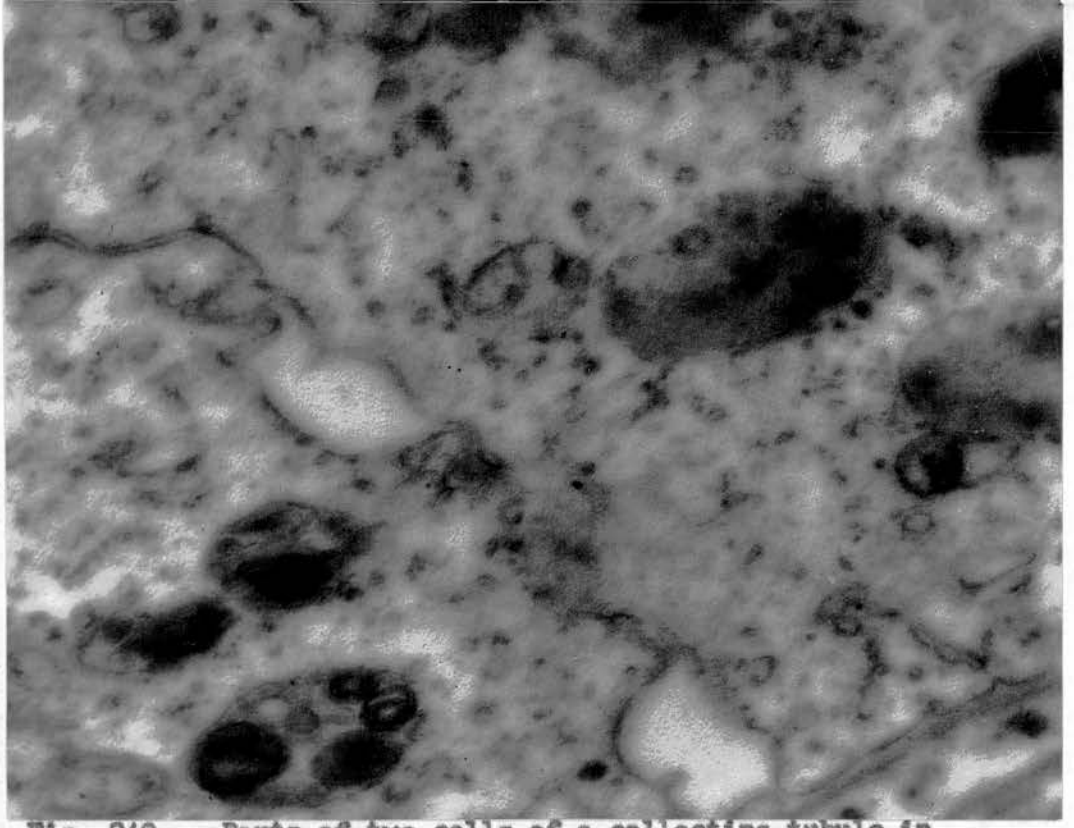


Fig. 240. Parts of two cells of a collecting tubule in the inner medulla of a six week potassium deficient rat. The structureless dense bodies appearing within the mitochondria are clearly seen. x 45,000

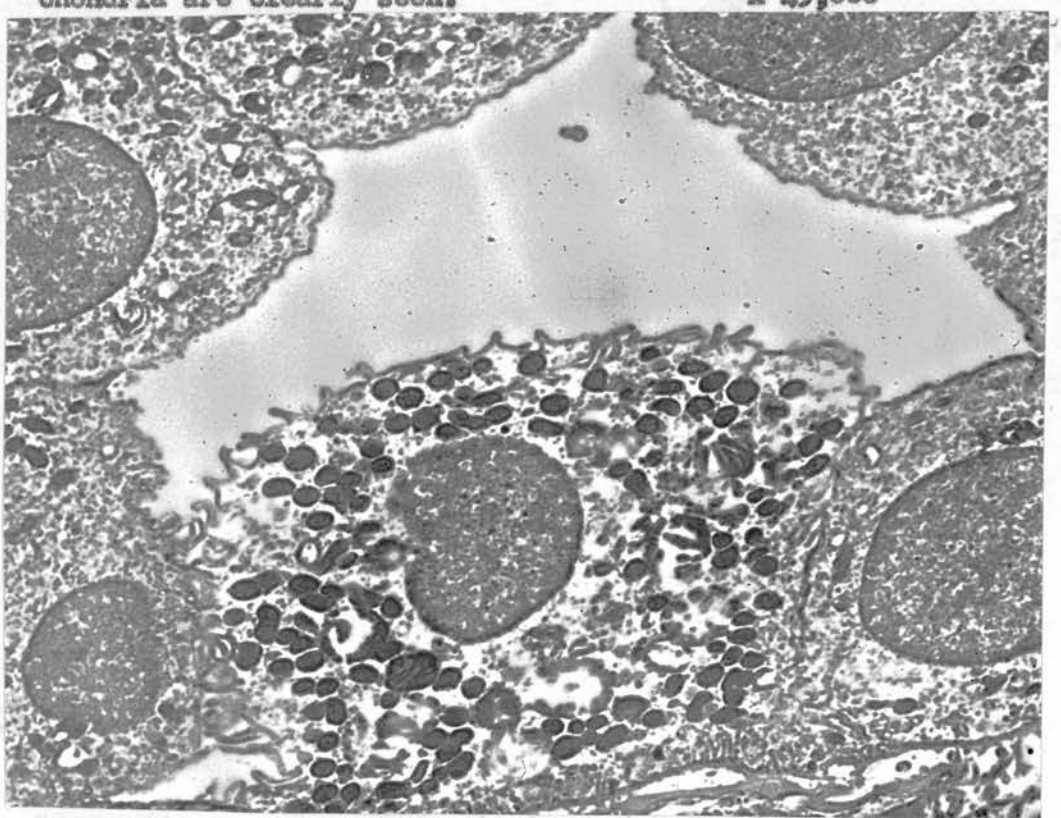


Fig. 241. A degenerate cell in an inner medullary collecting tubule of a six week potassium deficient rat. Note the abundance of the dark granules in the rarified cytoplasm. x 4,000

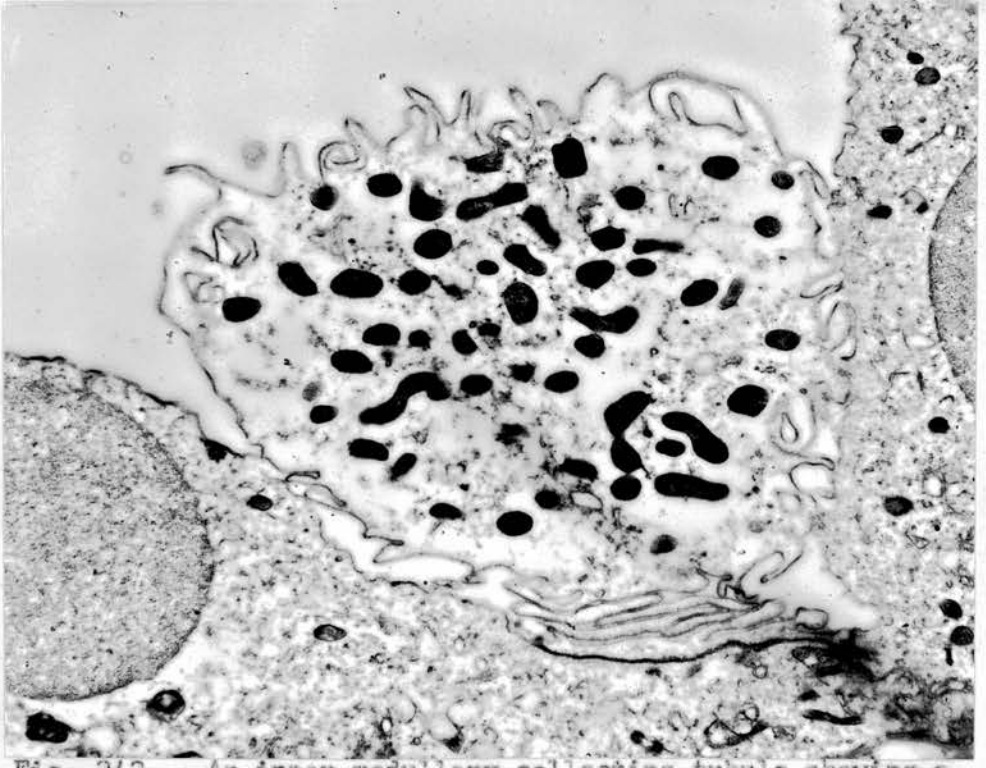


Fig. 242. An inner medullary collecting tubule showing a cell in a more advanced degree of degeneration than that seen in Fig. 241. The degenerate cell has withdrawn its processes from the neighbouring cells and is being shed off into the lumen. From a six week potassium deficient rat.
x 12,000

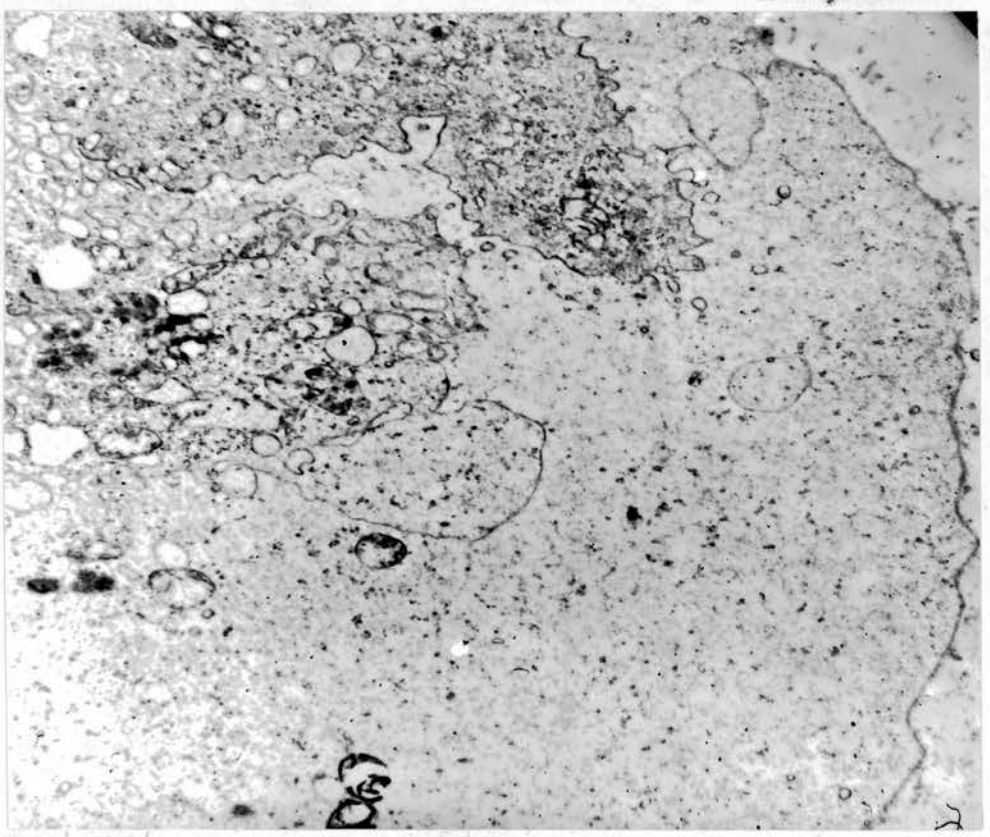


Fig. 243. An inner medullary collecting tubule with all its lining cells completely degenerate. From a six week potassium deficient rat. x 9,000

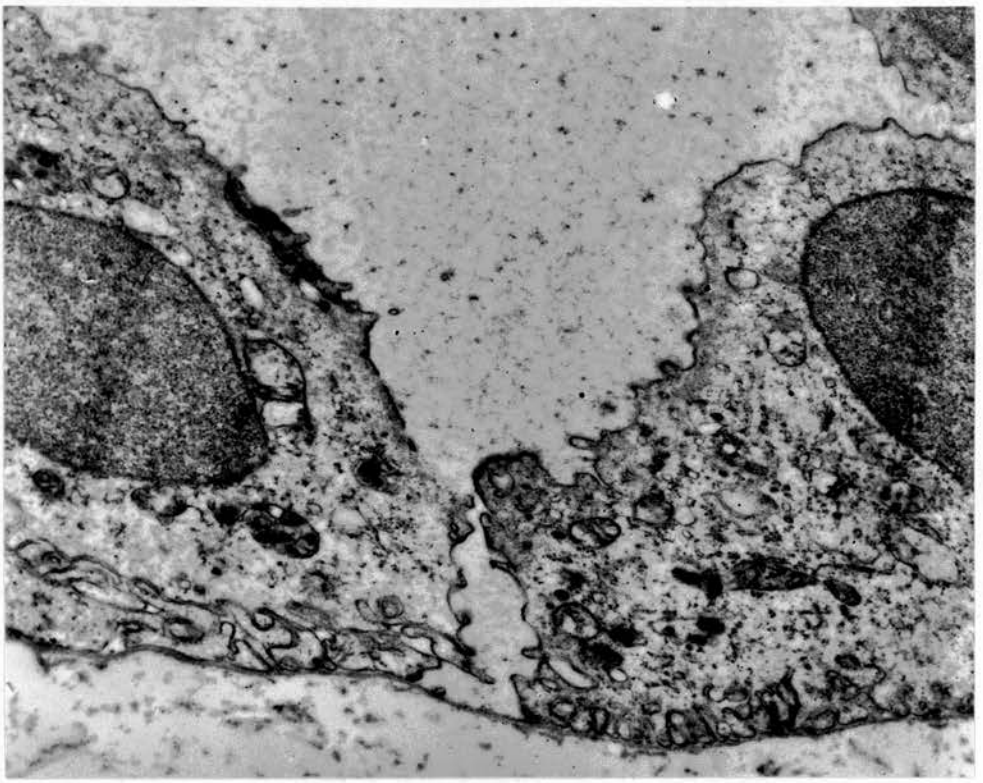


Fig. 244. Two cells of an inner medullary collecting tubule of a six week potassium deficient rat. Note the lateral separation starting from the basement membrane side. x 12,000

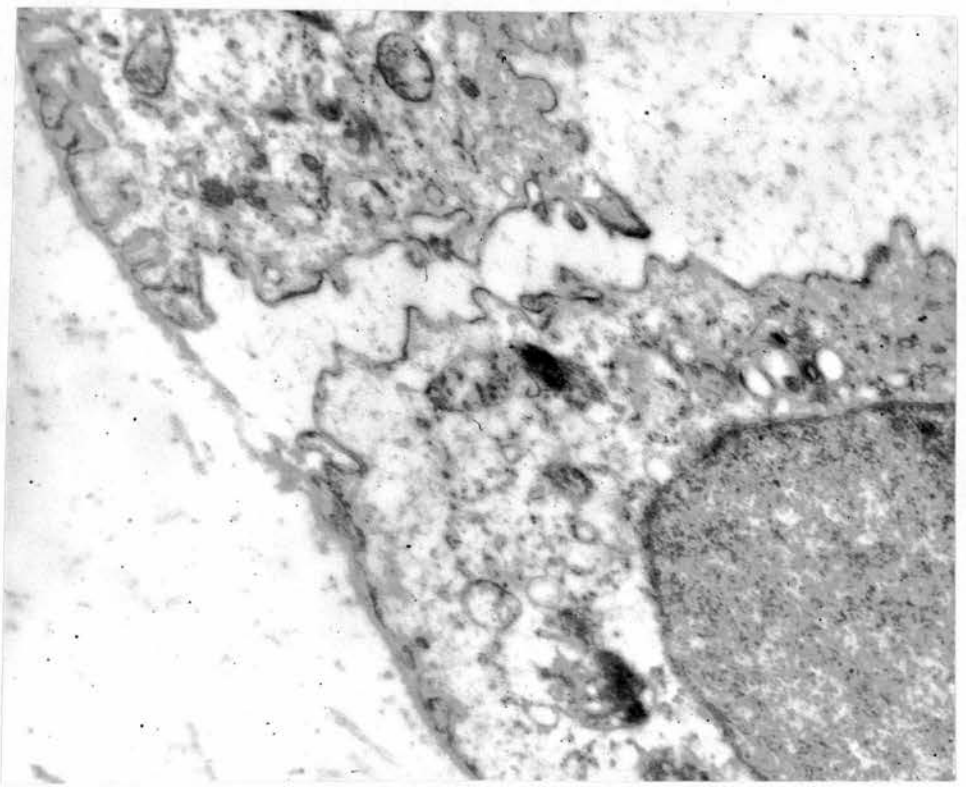


Fig. 245. Complete lateral separation between the cells of an inner medullary collecting tubule from a six week potassium deficient rat. x 24,000

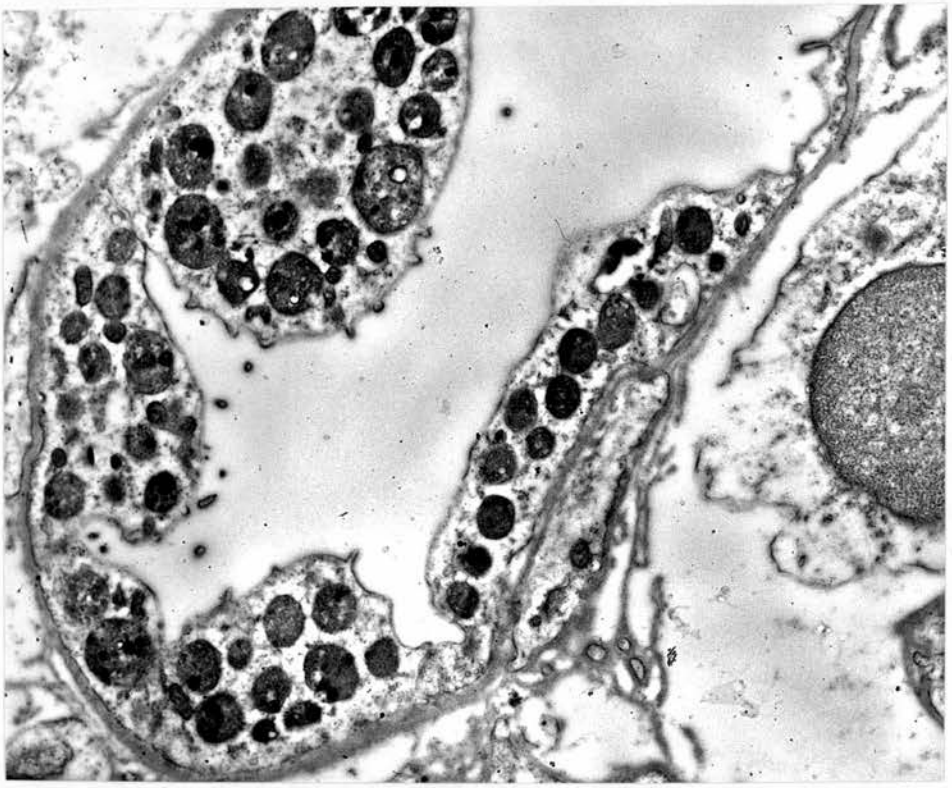


Fig. 246. Capillary in the inner medulla of a six week potassium deficient rat. The endothelium is swollen and is full of a large number of swollen, vacuolated mitochondria. x 9,000

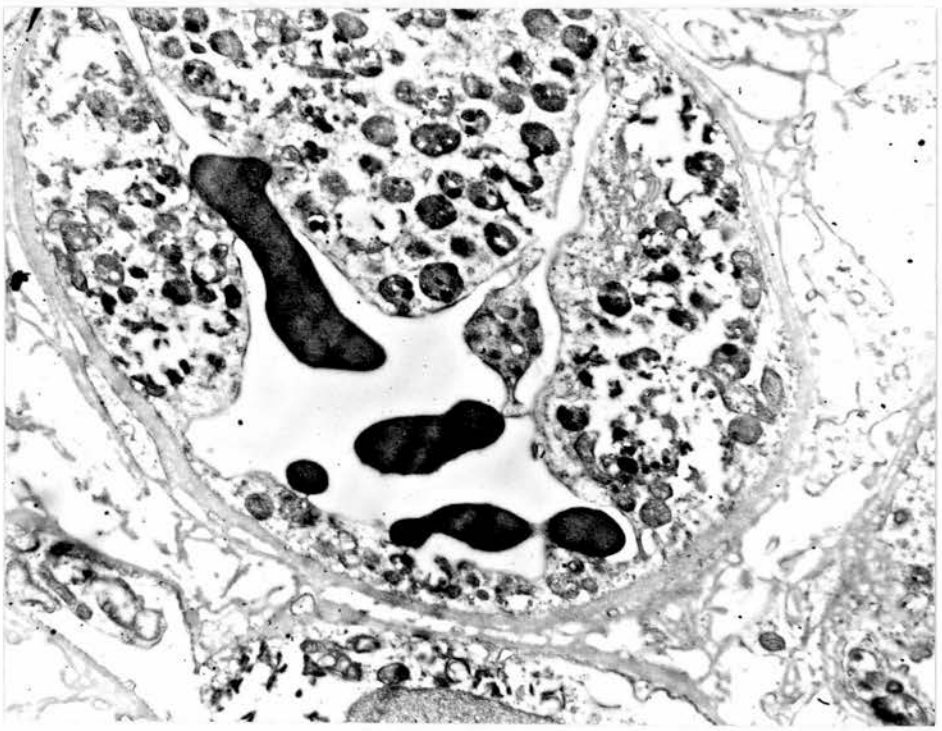


Fig. 247. An inner medullary capillary from a 6-week potassium-deficient rat. The endothelium is very swollen and is full of numerous, swollen, vacuolated mitochondria. x 6,000

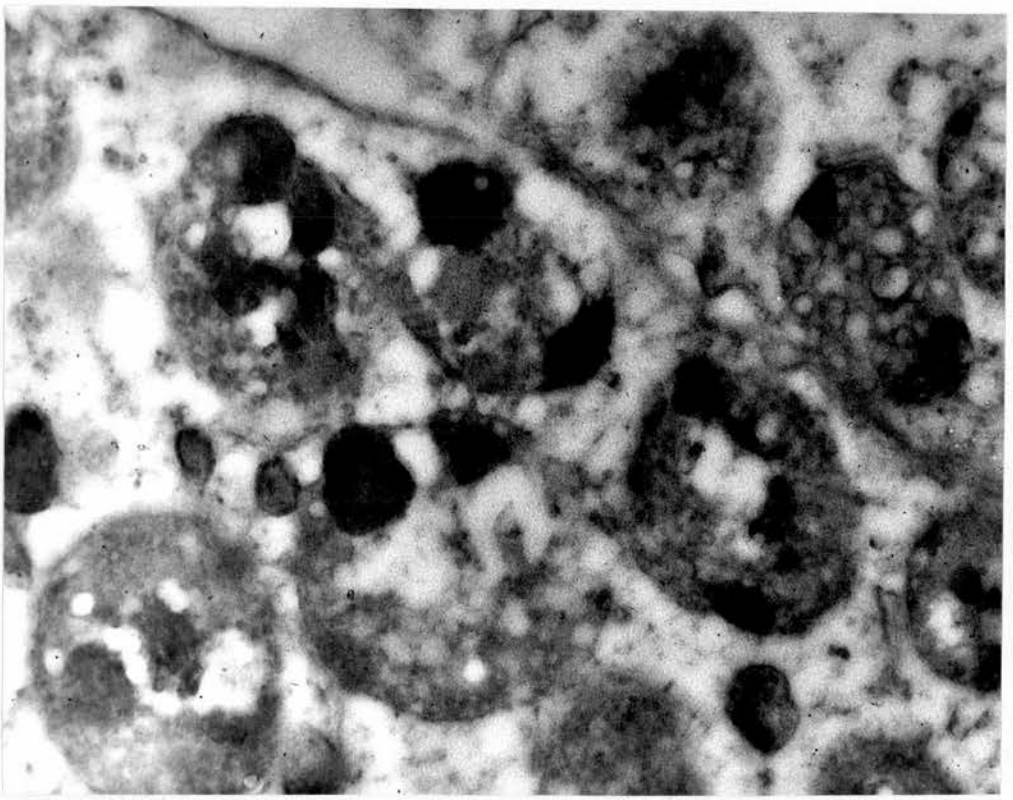


Fig. 248. Mitochondria in the endothelium of a capillary in the inner medulla of six week potassium deficient rat. Note that they are swollen, vacuolated and contain dense structureless osmiophilic bodies. x 45,000

All the other structures of the kidney were absolutely normal; glomeruli, convoluted part of the proximal tubules, thick segment of the loops of Henle, distal convoluted tubules, arterioles and larger arteries in the kidney.

Partially supplemented rats (Group D)

One rat showed no abnormality. The other, showed thickening and fibrillation of the basement membrane of the pars recta of the proximal tubules as well as thickening and fibrillation of the basement membrane of the thin segment in some loops of Henle. The degree of thickening was much less than that seen in the two weeks depleted group and it appeared to affect mainly, or only the sharp bends of the basement membrane.

Fully supplemented rats (Group E)

Examination of the renal tissue from these animals showed no abnormality whatsoever: in particular, the basement membranes of thin segments and pars recta of the proximal tubules were normal.

DISCUSSION.

After two weeks potassium depletion the only lesions found in the kidney were in the basement membrane of the descending limb of the loop of Henle, whether this descending limb was the pars recta or the proximal tubule or the thin segment of the loop. With an increasing degree of potassium depletion, lesions begin to appear in the collecting tubules and in the vasa recta capillaries, in the inner medulla.

The lesion in the basement membrane of thin segment and pars recta of the proximal tubule has not been described before. Till now there have only

been two studies by the electron microscope on the kidney in K-deficient rats. The first report (59) is a very short abstract without any electron micrographs published. It mentioned that the lesions are localised to the mitochondria of the proximal convoluted tubules and consist of swelling and vacuolation. Though similar lesions were noticed in three two-week depleted rats, they have never been seen in any of the other two-week depleted rats or in the four or six-week depleted and I feel certain that they are not directly related to depletion of potassium per se.

The second electron microscopic study of the kidney in potassium depletion (84) described lesions in the renal papilla only. No study was reported for the cortex or the outer zone of the medulla. The lesions described were the appearance of dense droplets in large numbers in the cells of the collecting ducts, interstitial cells and capillaries. These droplets were claimed not to be related to mitochondria . The presence of many dark dense granuoles in the collecting tubule cells in the inner zone of the medulla was confirmed in this study, and it was shown that they develop within mitochondria. The dense granuoles in the interstitial cells are probably not mitochondrial in origin. They were observed in these potassium depleted rats, in control rats, as well as in many other conditions and are most probably not specific for depletion of potassium. No granules were observed in the capillary endothelium but a definite swelling and marked increase in the number of mitochondria was noticed in the endothelium of the inner medullary capillaries. These mitochondria are degenerate and might eventually change into dense granuoles in a similar manner to what happens in the degenerate mitochondria of the collecting tubules in the inner medulla.

Among the previous light microscopic studies only one appears to be complete. In a well controlled study, with pair feeding and chemical data on the degree of potassium deficiency, Oliver and his co-workers (66) have demonstrated by microdissection that the lesions of potassium deficiency in the rat affect all the collecting tubules uniformly, and that different lesions occur in separate portions of the collecting system. The granular lesion was found in the innermost zone of the medulla which was otherwise entirely normal, and extended out to the epithelium of the papilla, while hyperplasia affected the cells in the inner strip of the outer medulla, particularly the intercalated "dark" cells. These workers further noted that except for minor patchy degenerative changes in the midportion of the proximal convolution the remainder of the nephron was essentially normal. The findings reported from this electron microscopic study entirely support the findings of the above-mentioned workers with the only exception that the collecting tubule lesions, particularly the hyperplasia was noticed only in some and not all collecting tubules. The only constantly observed lesion in this study in the basement membrane of the descending limb of the loop of Henle, is apparently beyond the resolving power of the light microscope.

The lesion in the basement membrane of the descending limb of the loop of Henle is responsible in my view for the impairment of the power to concentrate the urine, which is constantly observed, very early in association with the depletion of potassium. Theoretically, impairment in urinary concentrating power can result in several ways:

1) By interfering with the hyperosmotic reabsorption of sodium "the sodium pump mechanism" in the ascending limb, which is essential to create

the high osmotic concentration in the interstitial fluid of the medulla (together with the countercurrent system).

2) By interfering with the countercurrent multiplier system in the loops of Henle which (together with the sodium pump mechanism) is essential for rendering the medulla hypertonic.

3) By impairing water reabsorption in the distal tubule where isotonicity is normally re-established by water transfer out of an initially hypotonic fluid.

4) By impairing the passive water reabsorption through the wall of the collecting ducts as they traverse the hypertonic medulla.

The third possibility can be reasonably excluded since (a) micropuncture studies have shown that in potassium depleted rats hypotonic urine is present in the initial portion of the distal tubule and isosmotic urine is found further along the convolution (35). (b) No lesion has been detected in the distal convoluted tubule by the electron microscope.

The first possibility can be excluded since (a) the hypotonicity in the early distal tubule (35) indicates that the hyperosmotic sodium reabsorption is not impaired in the terminal segment of the ascending limb of the loop of Henle. (b) Potassium deficient rats andhumans with impaired concentrating ability are able to dilute the urine normally in response to a water load (55,76). (c) N_o lesion has been detected in the thick segment of the loop by electron microscopy.

These facts indicate that reabsorption of sodium without water proceeds normally in the loop of Henle.

The choice is largely between the second and the fourth possibliities. I think that it is the change in the descending limb of loop of Henle, interfering with the countercurrent multiplier system in the loops and resulting in an inability to create a hyperosmotic milieu in the inner medulla, which is responsible for the impaired power of concentration rather than the collecting tubule lesions for the following reasons:-

a) Manitius and co-workers (55) have clearly demonstrated that potassium depletion in rats and in dogs produce a much lower concentration of sodium and urea in the papilla than that present in the papilla of normal animals. Since the creation of a hyperosmotic milieu in the papilla depends on the integrity of both the sodium pump mechanism and the countercurrent multiplier system together and since the former mechanism is intact in potassium depletion as shown above, it must be the other limb of the countercurrent multiplier system (the descending limb) which is at fault and is responsible for the inability to create the hyperosmotic medium in the inner medulla.

b) After two weeks potassium depletion all the rats showed a definite and constant change in the basement membrane of the thin segment and pars recta of the proximal tubules but no lesion was detected in the collecting tubules. The polyuria however, was noticed in all the animals in the first week. Therefore a disturbance in the functional performance of the countercurrent multiplier system rather than an interference with the passive reabsorption of water from the collecting ducts can be suggested as the main cause of the polyuria and dilute urine, in potassium depletion.

c) The fact that human beings and dogs do not show any lesion in their collecting tubules in potassium depletion while they lose the power to concentrate the urine is a further evidence against the suggestion (55,66)

that a lesion in the collecting tubules is responsible for this tubular functioning deficit.

The change in the basement membrane of the descending limb of the loop of Henle is a very early abnormality and is very sensitive to mild depletion of potassium; (it has been observed to commence in Group D which were on the borderline of potassium deficiency) and this agrees very well with the fact that the excretion of a large volume of dilute urine is a very early renal tubular defect in potassium depletion. The way by which thickening and fibrillation of the basement membrane of the descending limb of the loop of Henle disturbs the functional performance of the countercurrent multiplier system has been discussed in the last chapter.

The early changes observed in this experiment in the descending limb of the loop of Henle are exactly similar to those observed after a single forcible hydration of the rats, described in the previous chapter, and raise a very important question. Does potassium depletion really impair the power of the kidney to concentrate urine or does it primarily stimulate the thirst centre, produce polydypsia and secondarily the excretion of a large volume of dilute urine? Most investigators concluded that it affects the concentrating power of the kidney simply by being unable to produce a concentrated urine, in their patients or experimental animals, by the injection of pitressin or by dehydration for 18-24 hours in most instances. In one of the best conducted and controlled experimental studies on the renal tubular defect of water reabsorption associated with depletion of potassium (41), where the deficient rats were dehydrated for 36 hours, the possibility of a primary polydypsia could not be excluded. In another well controlled study (4), potassium deficient rats were shown to drink more

than control rats and when they were dehydrated for 48 hours there was no incapacity of the kidney to concentrate the urine. In the experiments reported in the previous chapter, it was shown that though dehydration of the rats for 50 hours after a single dose of forcible hydration, reversed the changes in the basement membrane of the descending limb of the loop of Henle, the reversion was not complete. Shorter periods of dehydration, as used by most investigators, can easily be conceived to be unable to revert this morphological change in the basement membrane, which apparently has a high functional significance, especially as the rats depleted of potassium have been drinking excessively for several weeks and not just once. I believe therefore that the primary cause of the increased water turnover in potassium deficiency is polydypsia rather than loss of the power to concentrate the urine.

The degree of polydypsia and water turnover in the potassium deficient rats was found to be further augmented by removal of sodium from the potassium deficient diet, while the water turnover of these deficient rats drinking saline was only slightly greater than that of the saline drinking controls (4). The thirst stimulus in potassium depletion has been ascribed largely to a reduction of the chloride or extracellular fluid space (4).

The lesions found in the collecting tubules can explain many other tubular abnormalities that develop later in chronic potassium depletion. Increased urinary ammonia (58) has been reported. If ammonia production was one of the functions of the "dark cells" of the collecting tubules, we have an anatomical basis in the marked proliferation of these cells, that explains such state of affairs.

An exchange of Na^+ for H^+ is one of the active functions of the cells

of the collecting tubules (88). If this function is performed normally by the "light cells" a lesion in the mitochondria of these cells leading eventually to their replacement by dense structureless granules and then to degeneration of the cell, can result in an impairment of this function. In chronic potassium depletion in rats and dogs an excessive amount of sodium loss in the urine has been demonstrated (55). This faulty conservation of sodium might be related to such lesions in the mitochondria of the "light" cells. This active reabsorption of Na^+ from the medullary collecting tubules has been suggested to be of importance in maintaining the hyperosmolarity of the medullary interstitium (88). This inability to reabsorb sodium will be responsible for further diminishing the hyperosmolarity of the medulla and might explain the secondary or the tertiary phase of polyuria.

Chronic depletion of potassium is frequently associated with mild proteinuria, which has been found by electrophoresis of the urine to be of tubular origin (64). Degeneration, necrosis and shedding off of the cells of the collecting ducts easily explains this tubular proteinuria. Also, chronic potassium depletion has been found in humans and in animals to predispose to pyelonephritis. Obstruction of the collecting tubules by the proliferating cells which leads to proximal dilatation and internal hydronephrosis can easily explain such increased susceptibility of the potassium deficient kidney to pyelonephritis.

Finally, the lesions found in the capillaries of the vasa recta are very remarkable and have not been desdribed before. These lesions apparently impair the normal function of the vasae rectae as countercurrent exchangers which tend to maintain the hyperosmolarity of the papilla. On the other hand, they might be the result of a diminished osmotic concentration around

them created primarily by the lesions in the basement membrane of the descending limb of the loop of Henle. However, the absence of these capillary lesions after forcible hydration is in favour that they are genuine abnormalities related to depletion of potassium and that they play a role in the pathogenesis of the excretion of a large volume of dilute urine in kaliopenia.

REFERENCES.

1. Achor, R.W.P. and Smith, L.A.
Nutritional deficiency syndrome with diarrhoea resulting in hypopotassoemia, muscle degeneration and renal insufficiency; Report of a case with recovery.
Proc. Staff. Meet. Mayo Clinic, 30: 207, 1955.

2. Barrett, P.K.M., Bayliss, R.I.S. and Rees, J.R.
Conn's syndrome.
Proc. Roy. Soc. Med., 51: 720, 1958.

3. Barter, F.C. and Biglieri, E.G.
Primary aldosteronism: clinical staff conference at the National Institute of Health.
Ann. Int. Med., 48: 647, 1958.

4. Brokow, A.
Renal hypertrophy and polydipsia in potassium-depleted rats.
Amer. J. Physiol., 172: 333, 1952.

5. Brooks, R.V., McSwiney, R.R., Prunty, F.T.G. and Wood, F.J.Y.
Potassium deficiency of renal and adrenal origin.
Amer. J. Med., 23: 391, 1957.

5a. Campbell, C.H., Nicolaides, N. and Steinbeck, A.W.
Adrenocortical tumour with hypokalaemia and flaccid muscle paralysis.
Lancet, 2: 553, 1956.

6. Case records of the Massachusetts General Hospital. // 40081.
New Englan. J. Med., 250: 334, 1954.

7. Case records of the Massachusetts General Hospital. // 41171.
New Englan. J. Med., 252: 723, 1955.

8. Case records of the Massachusetts General Hospital. / 44491.
New Englan. J. Med., 259: 1128, 1958.

9. Chalmers, J.M., Fitzgerald, M.G., James, A.H. and Scarborough, H.
Conn's syndrome with severe hypertension.
Lancet, I: 127, 1956.

10. Ch'in, K.Y. and Hu, C.H.
Pathological lesions caused by B. dysenteriae.
A study of 89 autopsied cases.
Chinese Med. J. (Suppl. 3), 120, 1940.

11. Clarke, E., Evans, B.M., MacIntyre, I. and Milne, M.D.
Acidosis in experimental electrolyte depletion.
Clin. Sci., 14: 421, 1955.

12. Conn, J.W.
Primary aldosteronism.
J. Lab. & Clin. Med., 45: 661, 1955.

13. Conn, J.W. and Louis, L.H.
Primary Aldosteronism, a new clinical entity.
Ann. Int. Med., 44: 1, 1956.

14. Craig, J.M. and Schwartz, R.
Histochemical study of the kidney of rats fed diets deficient in potassium.
Arch. Path., 64: 245, 1957.

15. Crane, M.G., Short, G. and Peterson, J.E.
Observations on a case of primary aldosteronism.
Amer. J. Med., 24: 313, 1958.

16. Crane, M.G., Vogel, P.J. and Richland, K.J.
Observations on a presumptive case of primary aldosteronism.
J. Lab. & Clin. Med., 48: 1, 1956.

17. Darford, H.G. and Herrin, R.C.
Some physiological effects of salt restriction in the rat.
Amer. J. Physiol., 165: 128, 1951.

18. Darmady, E.M. and Stranack, F.
Microdissection of the nephron in disease.
Brit. Med. Bull., 13: 21, 1957.

19. de Graff, J., Schuurs, M.A.M.
Severe potassium depletion caused by the abuse of laxatives.
Acta Med. Scand., 166: 407, 1960.

20. Dunning, M.F. and Plum, F.
Potassium depletion by enemas.
Amer. J. Med., 20: 789, 1956.

21. Durlacher, S.H., Darrow, D.C. and Winternitz, M.C.
Effect of low potassium diet and of desoxycorticosterone acetate upon renal sizes.
Amer. J. Physiol., 136: 346, 1942.

22. Dustan, H.P., Corcoran, A.C. and Page, I.H.
Renal function in primary aldosteronism.
J. Clin. Invest., 35: 1357, 1956.

23. Eales, L. and Linder, G.C.
Primary aldosteronism. Some observations on a case in a Cape Coloured woman.
Quart. J. Med., 25: 539, 1956.

24. Earle, D.P., Sherry, S., Eichna, L.W. and Conar, N.T.
Low potassium syndrome due to defective renal tubular mechanism for handling potassium.
Amer. J. Med., 11: 283, 1951.

25. Evans, B.M., MacIntyre, I., Macpherson, C.R. and Milne, M.D.
Alkalosis in sodium and potassium depletion (with special reference to organic acid excretion).
Clin. Sci., 16: 53, 1957.

26. Fine, D., Meiselas, L.E., Colsky, J. and Oxenhorn, S.
Primary aldosteronism: Report of a case and discussion of the pathogenesis.
New Englan. J. Med., 256: 147, 1957.

27. Follis, R.H.
The pathology of nutritional disease: Physiological and morphological changes which result from deficiencies of the essential elements, aminoacids, vitamins and fatty acids.
Springfield, Illinois, Thomas, 1948. p. 30.

28. Follis, R.H., Orent-Keiles, E. and McCollum, E.V.
The production of cardiac and renal lesions in rats by a diet extremely deficient in potassium.
Amer. J. Path., 18: 29, 1942.

29. Follis, R.H.
Histochemical effects in rats resulting from adding rubidium or cesium to a diet deficient in potassium.
Amer. J. Physiol., 138: 246, 1943.

30. Fourman, P., McCance, R.A. and Parker, R.A.
Chronic renal disease in rats following temporary deficiency of potassium.
Brit. J. Exper. Path., 37: 40, 1956.

31. Giebisch, G. and Lozano, R.
The effect of adrenal steroids and potassium depletion on the elaborations of an osmotically concentrated urine.
J. Clin. Invest., 38: 843, 1959.

32. Glimstedt, G., Hakansson, R., Jonsson, N, Lundh, B. and Rorsman, H.R.
The collecting tubules of the kidney in the rat in dietary sodium and potassium deficiency.
Kungl. Fysiogr. Sallsk. Lund For handl, 23: 1, 1953.

33. Gordillo, G., Lapez, E. and Soto, R.
Tubular nephropathy casued by prolonged potassium deficiency.
Bol. Med. Hosp. Infant. (Mex), 16: 494, 1959.

34. Gottschalk, C.W. and Mylle, M.
Micropuncture study of the mammalian urinary concentrating mechanism: Evidence for the countercurrent hypothesis.
Amer. J. Physiol., 196: 927, 1959.

35. Gottschalk, C.W., Mylle, M., Winters, R.W. and Welt, L.G.
Micropuncture study of the osmolarity of renal tubular fluid in potassium depleted rats.
J. Clin. Invest., 37: 898, 1958.

36. Hellem, A.J.
Primary aldosteronism (Report of a case).
Acta med. Scandinav., 155: 271, 1956.

37. Henrikson, H.W.
Effect of potassium deficiency on gastrointestinal motility in rats.
Amer. J. Physiol., 164: 263, 1951.

38. Hewlett, J.S., McCullagh, E.P., Ferrell, G.L., Dusten, H.P., Poutasse, E.F. and Proudfit, W.L.
Aldosterone-producing tumours of the adrenal gland: Report of three cases.
J. Amer. Med. Ass., 164: 719, 1957.

39. Hollander, W. Jr.
The effect of potassium depletion on the kidneys.
North Carolina Med. J., 18: 505, 1957.

40. Hollander, W. Jr., Winters, R.W., Bradley, J., Holliday, M., Williams, T.F., Oliver, J. and Welt, L.G.
Effect of potassium repletion on the renal structural changes and the renal concentrating defect produced by potassium depletion in rats.
Amer. J. Med., 25: 123, 1958.

41. Hollander, W. Jr., Winters, R.W., Williams, T.F., Bradley, J., Oliver, J. and Welt, L.G.
Defect in the renal tubular reabsorption of water associated with potassium depletion in rats.
Amer. J. Physiol., 189: 557, 1957.

42. Holten, C. and Petersen, V.P.
Malignant hypertension with increased secretion of aldosterone and depletion of potassium.
Lancet, 2, 918, 1956.

43. Iacobellis, M., Muntwyler, E. and Griffin, G.E.
Enzyme concentration changes in the kidneys of protein and/or potassium deficient rats.
Amer. J. Physiol., 178: 477, 1954.

44. Jaffe, R.H. and Steinberg, H.
Über die vakuolare Nierendegeneration bei chronischer Ruhr.
Virchows Arch. f. path. Anat., 227: 313, 1919-1920.

45. Jensen, E.J., Baggenstoss, A.H. and Bargen, J.A.
Renal lesions associated with chronic ulcerative colitis.
Amer. J. Med. Sc., 219: 281, 1950.

46. Keye, J.D. Jr.
Death in potassium deficiency: report of a case including morpholotic findings.
Circulation, 5: 766, 1952.

47. Kjerulf-Jensen, K., Krarup, N.B. and Warming-Larsen, A.
Persistent hypokalaemia requiring constant potassium therapy.
Lancet, I: 372, 1951.

48. Kornberg, A. and Endicott, K.M.
Potassium deficiency in the rat.
Amer. J. Physiol., 145: 291, 1946.

49. Kretchmer, N., Dickenson, A., and Karl, R.
Aldosteronism in a nine-year old child.
Amer. J. Dis. Child., 94: 452, 1957 (Abst.)

50. Kulka, J.P., Pearson, C.M., and Robbins, S.L.
Distinctive vacuolar nephropathy associated with intestinal disease.
Amer. J. Path., 26: 349, 1950.

51. Liebow, A.A., McFarland, W. and Tennant, R.
The effects of potassium deficiency on tumour-bearing mice.
Yale J. Biol. & Med., 13: 523, 1941.

52. Luft, R., Ringertz, N. and Sjögren, B.
Two cases of cryptogenic hypokalaemia with pathological anatomical findings.
Acta endocrinol., 7, 196, 1951.

53. Mader, I.J. and Iseri, L.T.
Spontaneous hypopotassaemia, hypomagnesoemia, alkalosis and tetany dueto hyper-secretion of corticosterone-like mineralocorticoid.
Amer. J. Med., 19: 976, 1955.

54. Mahler, R.F. and Stanbury, S.W.
Potassium losing renal disease; renal and metabolic observations on a patient substaining renal wastage of potassium.
Quart. J. Med., 25: 21, 1956.

55. Manitius, A., Levitin, H., Beck, D. and Epstein, F.H.
On the mechanism of impairment of renal concentrating ability in potassium deficiency.
J. Clin. Invest., 39: 684, 1960.

56. Meyer, J.H., Gunert, R.R., Zepplin, M.T., Grumner, R.H., Bohstedt, G. and Phillips, P.H.
Effects of dietary levels of sodium and potassium on growth and on concentration in blood plasma and tissue of the white rat.
Amer. J. Physiol., 162: 182, 1960.

57. Milne, M.D., Muehrcke, R.C., and Aird, I.
Primary aldosteronism.
Quart. J. Med., 26: 317, 1957.

58. Milne, M.D., Muehrcke, R.C. and Heard, B.E.
Potassium deficiency and the kidney.
Brit. Med. Bull., 13: 15, 1957.

59. Muehrcke, R.C. and Bonting, S.L.
Electronmicroscopic and ultramicrobiochemical studies of the potassium depleted kidney.
Clin. Res., 6: 413, 1958 (Abstr)

60. Muehrcke, R.C. and Milne, M.D.
Primary aldosteronism, long-standing potassium depletion and pyelonephritis.
Clin. Res. Proc. 5: 190, 1957 (Abstr.)

61. Muntwyler, E. and Griffin, G.E.
Creatinine clearance in normal and potassium deficient rats.
Amer. J. Physiol., 173; 145, 1953.

62. Muntwyler, E. and Griffin, G.E.
Tissue electrolyte content of potassium and protein-deficient rats.
Proc. Soc. Exper. Biol. & Med., 89: 349, 1955.

63. Muntwyler, R., Iacobellis, M. and Griffin, G.E.
Kidney glutaminase and carbonic anhydrase activities and renal electrolyte excretion in rats.
Amer. J. Physiol., 184: 83, 1956.

64. Northam, B.E.
The Proteinuria of diabetic ketosis.
Presented at the Autumn Meeting of the Medical and Scientific Section of the British Diabetic Association, Birmingham 29th & 30th September, 1961.

65. Odessky, L. and Burdison, W.R.
Intestinal lipodystrophy (Whipple's disease) occurring with parathyroid hyperplasia and nephrosis.
Arch. Path., 49: 307, 1950.

66. Oliver, J., MacDowell, M., Welt, L.G., Holliday, M.A., Hollander, W. Jr., Winters, R.W., Williams, T.F. and Segar, W.E.
The renal lesions of electrolyte imbalance.
I. The structural alterations in potassium depleted rats.
J. Exper. Med., 106: 563, 1957.

67. Pearse, A.G.E. and Macpherson, C.R.
Renal histochemistry in potassium depletion.
J. Path. Bact., 75: 69, 1958.

68. Perkins, J.G., Peterson, A.B. and Riley, J.A.
Renal and cardiac lesions in potassium deficiency due to chronic diarrhoea.
Amer. J. Med., 8: 115, 1950.

69. Perude, H.S. and Phillips, P.H.
Effect of high fat diet on the potassium deficiency syndrome in the rat.
Proc. Soc. Exper. Biol. & Med., 81: 405, 1952.

70. Pollak, V.E., Flagg, G.W., Muehrcke, R.C. and Kark, R.M.
Potassium depletion following self-induced diarrhoea and vomiting treated by prolonged psychotherapy.
Clin. Res. Proc., 5: 194, 1957.

71. Ragan, C., Ferrekee, J.W., Phyfe, P., Atchley, D.W. & Loeb, R.F.
A syndrome of polydipsia and polyuria induced in normal animals by desoxycorticosterone acetate.
Amer. J. Physiol., 131: 73, 1940.

72. Relman, A.S., Lambie, A.T., Burrows, B.A. & Roy, A.M.
Cation accumulation by muscle tissue.
J. Clin. Invest., 36: 1249, 1957.

73. Relman, A.S. and Schwartz, W.B.
The nephropathy of potassium depletion; a clinical and pathological entity.
New Englan. J. Med., 255: 195, 1956.

74. Relman, A.S. and Schwartz, W.B.
The kidney in potassium depletion.
Amer. J. Med., 24: 764, 1958.

75. Richter, H.S.
Action of antidiuretic hormone in potassium depleted rats; relation to aldosteronism.
Proc. Soc. Exper. Biol. & Med., 97: 141, 1958.

76. Rubini, M.E.
Water excretion in potassium deficient man.
J. Clin. Invest., 40: 2215, 1961.

77. Russell, G.F.M., Marshall, J. and Stanton, J.B.
Potassium-losing nephritis: a clinical investigation.
Scott. Med. J., 1; 122, 1956.

78. Schrader, G.A., Prickett, C.O. and Salmon, W.D.
Symptomatology and pathology of potassium and magnesium deficiencies in the rat.
J. Nutrition, 14: 85, 1937.

79. Schwartz, W.B. and Relman, A.S.
Metabolic and renal studies in chronic potassium depletion resulting from overuse of laxatives.
J. Clin. Invest., 32: 258, 1953.

80. Siebenmann, R.E.
Über eine tödlich verlaufende Anorexia nervosa mit Hypokaliamie.
Schweiz. med. Wschr., 85: 468, 1955.

81. Skanse, B., Möller, F., Gydell, K., Johansson, S. and Wulff, H.B.
Observations on primary aldosteronism.
Acta med. Scandinav., 158: 181, 1957.

82. Smith, S.G., Black-Shaffer, B. and Lasater, T.E.
Potassium deficiency syndrome in the rat and the dog.
Arch. Path., 49: 185, 1950.

83. Spargo, B.
Kidney changes in hypokalaemic alkalosis.
J. Lab. & Clin. Med., 43: 802, 1954.

84. Spargo, B., Straus, F. and Fitch, F.
Zonal renal papillary droplet change with potassium depletion.
Arch. Path., 70: 599, 1960.

85. Stanbury, S.W. and Macaulay, D.
Defects of renal tubular function in the nephrotic syndrome.
Quart. J. Med., 26: 7, 1957.

86. Tauxe, W.N., Wakim, K.G. and Baggenstoss, A.H.
The renal lesions in experimental deficiency of potassium.
Amer. J. Clin. Path., 28: 221, 1957.

87. Thomas, R.M., Mylon, E. and Winternitz, M.C.
Myocardial lesions resulting from dietary deficiency.
Yale J. Biol. & Med., 12: 345, 1940.

88. Ullrich, K.J.
Function of the collecting ducts.
Circulation, 21: 869, 1960.

89. Van Buchem, F.S.R., Doorenbos, H. and Elings, H.S.
Primary aldosteronism due to adrenocortical hyperplasia.
Lancet, 2: 335, 1956.

90. Van Buchem, F.S.R., Doorenbos, H. and Elings, H.S.
Conn's syndrome, caused by adrenocortical hyperplasia.
Pathogenesis of the signs and symptoms.
Acta endocrinol., 23: 313, 1956.

91. Wachstein, M. and Meisel, E.
Enzymatic staining reactions in the kidneys of potassium depleted rats.
Amer. J. Path., 35: 1189, 1959.

92. Welt, L.G., Hollander, W. Jr., and Blythe, W.B.
The consequences of potassium depletion.
J. Chronic Dis., 11: 213, 1960.

93. Wigley, R.D.
Potassium deficiency in anorexia nervosa, with reference to renal tubular vacuolation.
Brit. Med. J., 2: 110, 1960.

94. Williams, R.H. and MacMahon, H.E.
Gastroenterocolitis: carcinoma of pancreas: "Clear-cell" nephrosis.
Bull. New Englan. Med. Center, 9: 274, 1947.

95. Wirz, H.
The location of antidiuretic action in the mammalian kidney: In The neurohypophysis , London 1957, Heller, H. Editor, Academic Press Inc., p. 157.

96. Woods, J.W., Welt, L.G. and Hollander, W. Jr.
Susceptibility of rats to experimental pyelonephritis during potassium depletion.
J. Clin. Invest., 40: 599, 1961.

97. Woods, J.W., Welt, L.G., Hollander, W. Jr., and Newton, M.
Susceptibility to experimental pyelonephritis during and after potassium depletion.
J. Clin. Invest., 38: 1056, 1959.

98. Wu-Hao-Tu, Jones, C.C. and Allen, M.S.
Nephropathy of potassium depletion. Report of a fatal case.
Ann. Int. Med., 53: 796, 1960.

99. Wyngaarden, J.B., Keitel, H.G. and Isselbacher, K.
Potassium depletion and alkalosis. Their association with hypertension and renal insufficiency.
New Englan. J. Med., 250: 597, 1954.

MAGNESIUM DEFICIENCY NEPHROPATHY.

MAGNESIUM DEFICIENCY NEPHROPATHY.

Introduction.

Every advance raises fresh problems, and advances in the techniques of fluid therapy have now made magnesium metabolism a matter of practical importance. Years ago patients died because they were not thirsty enough or because they were too weak to drink. Once this fact was realised, and they were given water, the problem of fluid therapy for the majority of them was solved. Some, however, with continuous losses of intestinal fluid, now survived to become deficient in sodium, and after a few days they would die of that. As sodium deficiency came to be recognised and treated, so more patients who would otherwise have died continued to lose fluid and, after a week or two, they died of potassium deficiency. Now this too, is treated and patients can survive long enough to become depleted of magnesium.

One reason why the possibility of magnesium deficiency was not widely recognised was the difficulty of its estimation. The available methods for the determination of magnesium in biological fluids and tissues were, until quite recently, either tedious or inaccurate, and plasma magnesium concentrations were estimated in clinical laboratories as rarely as was potassium before the advent of the flame photometer. Recently, however, the physical methods of flame spectrophotometry (2), cathode-ray polarography (20) and absorption spectrophotometry (9) have introduced accurate techniques to replace the older chemical methods of determining magnesium in biological work and reliable instruments are now commercially available.

The amount of magnesium in the body ranks with that of sodium and potassium: the amount of sodium is 4000 m. equiv., largely in the extracellular fluid; potassium is 3000 m. equiv., nearly all inside the cells; and magnesium is 2000 m. equiv. (11, 40). Like potassium, it is mainly an intracellular ion, but unlike potassium, at least half and perhaps three quarters of the magnesium in the body is in bone (12).

Magnesium is lost to the body by excretion in the faeces and urine; it is replinished from the diet, The amount of magnesium in a normal diet is of the order of 30 m. equiv.; it is present in nearly all foods. With an ordinary mixed diet, a deficiency of magnesium is unlikely (12). The magnesium excreted by the kidneys has probably all been filtered at the glomeruli and the amount in the urine represents what has escaped tubular reabsorption.

Though it has been known that clinical symptoms due to dietary deficiency in magnesium occur in dairy cows, little attention has been paid to the possible role of changes in the general magnesium balance or of abnormal fluxes of magnesium ions between the intracellular and extracellular fluids in the aetiology of human disease until very recently. The cow is the victim of two forms of magnesium deficiency; milk tetany encountered in calves reared beyond the natural time of weaning exclusively on a diet of milk, and grass staggers found in cows put out on certain pastures. Both forms of magnesium deficiency are ultimately the result of a poor absorption of magnesium from the gut. Magnesium deficiency in the cow has contributed most of our knowledge of the clinical syndrome of magnesium deficiency in humans. After it was suggested that magnesium deficiency might be observed clinically in patients, many reports have appeared in the literature in the last few years confirming the existence

of a syndrome of magnesium deficiency in human beings (7,10,16,33, 41). Recently, Hanna (15) has reviewed the causes of magnesium depletion in humans and emphasised that with the developmentof modern electrolyte therapy cases of hypomagnesoemia are being encountered more frequently. Magnesium depletion may be expected to become of clinical importance when a low intake of magnesium is associated with abnormal loss of gastro-intestinal secretion or an excessive loss of magnesium in the urine, or when continuous intestinal aspiration is combined with the infusion of magnesium-free fluids. Patients with intestinal fistulae, intractable diarrhoea, massive resection of small bowel, diabetic ketosis, and those in the diuretic phase of the recovery from acute renal insufficiency may become depleted of magnesium. Cases of primary aldosteronism (28,29), steatorrhoea, idiopathic hypercalcuria, thryotoxicosis, renal tubular acidosis, malignancy with bone metastases and excessive use of purgatives (15,16,18) have also been shown to have a negative magnesium balance. These recent clinical reports have revived the interest in the metabolism of this ion and in the study of the immediate effects and remote sequele of body depletion of magnesium.

In the study of magnesium deficiency, the usual experimental animals have been pressed into service: rats, mice, guinea-pigs, rabbits and dogs. In addition, the team included an unusual member, the cow, because spontaneous deficiency of magnesium in this animal creates an economic problem.

The first attempt to deprive an animal of magnesium appears to have been made by Osborne and Mendel in 1918 during the course of a general study of the inorganic elements in the nutrition of the rat (31). Their

"magnesium-free" diet, however, still contained enough magnesium to prevent any untoward effects. Acute magnesium deficiency, produced in both rats and dogs by a diet very low in magnesium has been studied by McCollum, Kruse and co-workers and described in a series of papers (21,22,23,26).

The main features of the experiments on rats, were the production of acute hyperaemia of the skin and loss of hair, convulsions and death. The results have been confirmed by other workers (24,36), and according to Tufts and Greenberg (38) two phases are observed in magnesium deficiency in rats. The first is marked by vasodilatation, hyperaemia and hyper-excitability, and the second by the development of nutrition failure, cachexia and kidney damage.

The literature on the gross and microscopic pathological alterations associated with magnesium deficiency has been rather meagre. In 1932, Cramer found that most of the rats which he placed on a prepared synthetic diet, low in magnesium, developed marked changes in the kidneys (8). At autopsy, the kidneys of many of these animals were found to be enlarged, had puckered surfaces, some showing a grey mottling. On cut section, they exhibited definite striations at the junction of the medulla and cortex. Microscopically, they showed a picture of extensive degeneration of tubules and glomeruli, which was most marked at the junction of the medulla and cortex, where, in some sections, the necrosis was accompanied by calcerous deposits in the tubules and glomeruli. In 1934, Brookfield (6) observed similar changes in the kidney of rats given a diet of low magnesium content but he stressed the finding that no calcification was observed. In 1937, Schrader and his co-workers (34) noted mild degenerative changes in the renal tubules of rats on diets low in magnesium; no calcification was noted. In the same year, Watchorn and McCance (39) reported that rats kept on a

low magnesium diet to induce a state of subacute deficiency showed after a period of 12 weeks, calcareous deposits throughout the cortex and medulla in the straight and collecting tubules producing obliteration and sometimes cystic dilatation of the tubules above the level of the cast. Glomerular changes or lesions indicative of any type of nephritis were stated to be completely absent. In 1938, Greenberg et al(13) showed that prolonged deprivation of magnesium in the rat eventually produced a nephrosis-like type of kidney degeneration, beginning by degenerative changes in the tubules and progressive calcification in the cortico-medullary zone and spreading later to the cortex. The lesions found were similar to those originally described by Cramer in 1932 but the degree of calcification was more marked The magnesium deficient animals showed persistent albuminuria, but no haematuria, and their serum proteins were definitely lowered. Similar degenerative changes followed by the deposition of calcareous material in the areas of degeneration were again reported in rat kidneys in 1944 (37) and in 1950 (25). In 1958, MacIntyre and Davidson (27) reported that magnesium deficiency in rats produced secondary potassium depletion, sodium retention, hypercalcaemia and nephrocalcinosis; nephrocalcinosis occurred only when hypercalcaemia was also present. In 1959, Hess et al (19) using histochemical methods for the determination of various dehydrogenase and diaphorase activities, have found intracellular alterations to occur in the rat kidney in the first days of dietary magnesium deficiency. This alteration was confined mainly to distal segments of the proximal convolutions and consisted of mitochondrial swelling followed by the appearance of lipid droplets. After 9 days, marked decrease of mitochondrial enzymic activity was found in the damaged areas initiating intracellular calcification and necrosis. A close relationship was shown to exist between a decrease of

plasma magnesium, an increase of plasma calcium and the mitochondrial alterations observed. Visible renal calcification was accompanied by a marked rise of kidney calcium content.

In 1938, Moore et al (30) studied the pathological changes in calves fed diets low in magnesium. In more than half their animals, thekidneys showed marked proliferation of fibroblasts and fibrosis of the interstitial tissue, marked tubular necrosis was also observed in one third of the cases. They stressed the comparatively slight degree of injury to the glomeruli in contrast with the marked injury of the interstitial and tubular tissues. However, in 1954, Blaxter, Rook and MacDonald (4) found no degenerative lesions in kidney of magnesium deficient calves.

The third animal in which deficiency of magnesium was reported to produce renal lesions is the rabbit. Barron and co-workers, in 1949, (3) described that in magnesium deficient rabbits, damage involved both the renal corpuscle and the tubules and was not uniform throughout the kidney but was limited to certain areas of the tissues. Changes were observed in both the cortex and medulla, which consisted of degeneration of the tubular epithelium and fibrosis of the corticomedullary region. The glomeruli within the intact Bowman's capsule were often displaced to the periphery by an amorphous acidophilic staining mass of material. Such renal corpuscles were often enlarged to as much as twice the diameter of controls.

No one has yet described any pathological changes attributable to magnesium deficiency in man, but the experimental findings in amimals suggests that some unexplained tissue infiltrations and some obscure instances of nephrocalcinosis might be the result of an episode of magnesium deficiency (25,27). This is one of the questions that may be resolved by a

systematic investigation of possible cases of magnesium deficiency in humans, including the study of renal biopsy material whenever possible.

Material and Methods.

Thirty female hooded rats "Sprague-Dawley" strain, initially weighing between 120 and 150 grams were used for the study. They were divided into three equal groups. The control group and the two experimental groups were pair fed on diets identical in all respects other than magnesium content.

The diet used was that described by MacIntyre and Davidson in 1958 (27). It consisted of the following:

Acid-washed casein	200 g.
Arachis oil	80 g.
Cod liver oil	20 g.
(to which had been added 50 mg. of vitamin E)	
Cane sugar	660 g.

To this basic diet 40 g. of salt mixture C was added for the control group or 36.7 g. salt mixture D for each of the two experimental groups.

Salt mixture C.

	NaCl	33 g.
	$CaCO_3$	188 g.
	$K H_2PO_4$	170 g.
	Potassium citrate	61 g.
	Ferric Citrate	7.5 g.
	Tracer mixture	1 g.
and	$MgCl_2\ 6H_2O$	41 g.

In Salt mixture D the magnesium chloride was omitted.

The tracer mixture consisted of:

KI	13 g.
NaF	10 g.
$MnCl_2 4H_2O$	2 g.
Cu_2Cl_2	0.5 g.

A stock vitamin solution was prepared and stored at -20°C, 250 ml. contained:

Vitamin B1	0.03 g.
Vitamin B6	0.03 g.
Biotin	0.006 g.
Pantothenol	0.3 g.
p-aminobenzoic acid	1.5 g.
Inositol	1.5 g.
Nicotinic acid (sodium salt)	1.5 g.
Choline chloride	4.5 g.
Folic acid	0.015 g.
Riboflavin	0.12 g.
Vitamin B12	0.0003 g.
Vitamin K	0.0006 g.
(Synkavit, Roche Products Ltd)	

This solution (16 ml.) made to two litres with water, provided sufficient drinking water and water soluble vitamins for 30 rats for 3 days.

The electrolyte content of the diets as found by analysis was:

Na		46 m.equiv/Kg.
K		141 m.equiv./Kg.
Ca		200 m.equiv./Kg.
Mg	"controlgroup"	36 m.equiv./Kg.
	"deficient group"	1 m.equiv./Kg.

Group A deficient rats were kept on the magnesium deficient diet for 13 days.

Group B deficient rats for 3 days and Group C control rats were kept on the control diet for 13 days.

At the end of the experimental period the rats from the three groups were anaesthetised by the intraperitoneal injection of 0.1 ml. pentobarbitone (Veterinary Nembutal, Abbott, London). Blood was collected from the abdominal aorta then the right kidney was immediately removed and prepared for microscopy, electron and light, as explained before, while the left kidney as well as muscles from the carcass collected for chemical analysis. In the light microscopic preparations, in addition to the routine stains used (H. & E. & PAS), sections were stained by Von Kossa and by Feulgen techniques.

The muscle, kidney and plasma were analysed for their magnesium and

calcium contents by the methods described by Alcock et al (2). Statistical analysis was carried out by the methods described by Snedecor in 1956 (35).

Results.

After about 11 days, the deficient rats in Group A developed vasodilatation and oedema of the nose, ears, and paws and showed signs of irritability in the last few days.

The results of the plasma, muscle and kidney analyses are shown in Table 11. These results confirm the finding of MacIntyre and Davidson (27). Little or no significant change was noted in the concentration of magnesium in the kidneys in the deficient rats. In the skeletal muscles, there was unequivocal evidence of progressive depletion of magnesium. The plasma magnesium fell from the control value of 1.48 to 0.59 m. equiv./l. after 13 days of depletion; the fall was also significant after 3 days. The only highly significant result in the calcium estimations was a marked increase in the calcium content in the kidneys of the deficient group, from the control value of 11.89 m.equiv./Kg. to 15.63 after 3 days and to 20.51 after 13 days of depletion of magnesium.

Light Microscopy.

As compared with the control group (Fig. 249) the kidneys from the two deficient groups showed definite abnormalities which were more marked after 13 days depletion of magnesium, but were also noticeable after 3 days.

The glomeruli and the blood vessels were entirely normal. There was a slight increase in the interstitial tissue in the 13 days deficient rats but this was apparently secondary to the tubular lesions.

TABLE 11.

Rat No.	Plasma m.equiv/l.		Muscle m.equiv/Kg. dry fat free solids		Kidney m.equiv/Kg.dry fat free solids	
Control Group	Mg^+	Ca^+	Mg^+	Ca^+	Mg^+	Ca^+
1 C	1.4	5.5	100.9.	10.1	83.8	2.6
2 C	1.3	5.4	98.8	11.4	84.6	12.8
3 C	1.3	5.4	99.3	10.3	82.9	10.2
4 C	1.6	5.4	100.5	10.6	83.8	11.7
5 C	1.4	5.6	100.4	10.2	84.1	11.8
6 C	1.5	5.4	99.9	11.0	83.6	12.0
7 C	1.5	5.2	100.1	11.1	83.7	11.8
8 C	1.7	5.2	98.3	10.2	83.4	11.5
9 C	1.5	5.6	99.4	10.5	84.2	12.6
10 C	1.6	0.4	98.9	10.6	83.3	12.1
Mean $\pm$ S.E.M.	1.48 ± 0.033	5.43 ± 0.021	99.76 ± 0.63	10.6 ± 0.4	83.77 ± 0.41	11.89 ± 0.3
Def.for 3 days						
1 B	0.8	5.7	94.6	10.2	82.8	13.5
2 B	0.9	5.1	95.7	10.2	80.9	15.6
3 B	0.8	5.4	96.3	10.1	82.1	15.7
4 B	0.8	5.0	96.2	9.6	80.9	21.2
5 B	0.9	5.0	97.1	10.7	81.6	12.2
6 B	0.8	5.4	96.0	10.2	81.7	14.0
7 B	0.9	5.1	96.4	10.4	81.9	17.2
8 B	0.8	5.3	95.6	9.9	81.8	17.1
9 B	0.8	5.2	96.1	10.0	80.9	14.9
10 B	0.9	5.3	95.9	10.4	32.0	16.3
Mean $\pm$ S.E.M.	0.83 ± 0.03	5.23 ± 0.23	95.97 ± 0.7	10.17 ± 0.3	81.77 ± 0.55	15.63 ± 2.81
Def.for 13 days						
1 A	0.6	6.3	86.4	12.6	80.9	32.6
2 A	0.7	5.7	90.2	11.9	81.4	12.9
3 A	0.5	5.8	88.3	13.0	81.6	13.6
4 A	0.6	5.6	86.2	9.2	83.8	10.2
5 A	0.6	5.8	91.4	9.6	79.2	42.5
6 A	0.6	5.7	94.2	11.7	82.4	15.6
7 A	0.5	5.7	86.7	16.2	80.6	16.2
8 A	0.6	5.8	89.1	12.3	31.4	23.8
9 A	0.6	5.7	88.3	14.1	82.6	20.5
10 A	0.6	5.6	86.1	10.3	80.2	17.2
Mean $\pm$ S.E.M.	0.59 ± 0.026	5.8 ± 0.087	89.06 ± 1.14	12.03 ± 0.89	81.41 ± 0.55	20.51 ± 4.6

The characteristic change was noticed in the tubules. Foci of dilated tubules were seen all over the cortex and outer medulla (Fig. 250). In the 3 days deficient animals these foci were mostly localised to the cortico-medullary zone, but in the 13 days deficient rats they were observed all over the cortex and outer zone of the medulla in addition to the cortico-medullary region. In this latter group, the degree of dilatation was more marked and the number and size of the foci was greater than in the 3 days deficient animals. The type of tubule which was dilated could not be always ascertained; some, however, had a definite though frequently interrupted brush border; indicating that they are proximal tubules (Fig. 251).

In Group B deficient animals, the lining cells of many of the slightly dilated tubules were swollen and were frequently vacuolated. As the tubules got more dilated their lining cells appeared to lose the brush border and to become flattened so that in the 13 days deficient rats, only a minority of the dilated tubules had cells with a vacuolated cytoplasm (Fig. 251).

At first sight, the dilatation of the tubules appeared as if it had resulted from a distally situated obstruction rather than a degeneration or atrophy of the tubular cells. However, careful examination failed to show any cause for the obstfuction, even in the 3-day deficient rats at the cortico-medullary region or in the outer zone of the medulla. No hypertrophy or proliferation of the lining cells and no intraluminal casts could be seen occluding the lumen in any of the tubules. However, after 13 days depletion, a few markedly dilated tubules contained in their lumina a deposit not unlike the nidus of a calculus, which appeared refractile and was thought to consist of necrotic cellular debris (Fig. 252). However, it reacted

negatively to both the Feulgen and the von Kossa stains.

The von Kossa stain showed in some dilated tubules after 13 days depletion of magnesium, a very fine tracery of silver-staining granules, sometimes localised to individual cells and particularly marked in the brush border when it had not yet completely disappeared.

Electron microscopy.

Group C. The kidneys from the control rats were entirely normal.

Group B. The glomeruli, the arteries and the capillaries from the 3-days deficient rats were normal.

Lesions were noticed only in the tubules, and were seen mainly in the blocks taken from the outer zone of the medulla and from the deeper part of the cortex. The tubules at the renal papilla; thin segments and collecting tubules, appeared quite normal.

The tubular changes were focal, and though some tubules were very abnormal others, sometimes immediately adjacent were quite normal.

The observed lesions affected principally the pars recta of the proximal tubules. The affected tubule was dilated and some of its lining cells showed loss of their microvilli resulting in an interruption of the brush border (Fig. 253). The cells also showed multiple small cytoplasmic vacuoles (Fig. 254) and few dense structureless cytoplasmic granuoles of various sizes, but usually several times the size of the mitochondrion (Fig. 254 and 255). More severely affected tubules were markedly dilated and their cells had scanty microvilli (Fig. 256), were moderately flattened and eventually lost all their microvilli over extensive stretches (Fig. 257).

The rest of the proximal tubule appeared normal, in particular the

basement membrane and the mitochondria looked normal.

Group A. The glomeruli and the blood vessels were normal. The thin and thick limbs of the loops of Henle were also normal (Fig. 258). The lesions were focal and affected mainly, if not entirely, the proximal tubules but were more widespread, all over the cortex and outer medulla, and more marked than in Group B. The fact that the lesions were occasionally noticed in some tubules, the type of which could not be identified even by electron microscopy is worth mentioning. Such tubules, encountered in the cortex and outer medulla, did not conform to the ideal description of either proximal, distal or collecting tubules (Fig. 259). My impression is that most of them are proximal tubules that have completely lost their microvilli, the most characteristic property for their identification, but I cannot be certain that similar changes to those affecting the proximal tubules in magnesium depletion do not affect, to some degree, a few distal and collecting tubules.

The changes noticed in the proximal tubules after 13-days depletion of magnesium were of the same nature as those observed after 3 days: dilatation of the tubules, vacuolisation of their lining cells, loss of the microvilli and the appearance of dense bodies in the cytoplasm (Fig. 260), the basement membrane and the mitochondria remaining normal.

The earliest abnormality to be noted in the least affected tubules appears to be dilatation of the lumen with little alteration in the cellular contents or the microvilli (Fig. 261). Vacuoles then appear in the cytoplasm (Fig. 262) and the microvilli begin to disappear (Fig. 263) and this gradual disappearance of the microvilli is accompanied by further dilatation of the lumen (Fig. 264), until finally all the microvilli disappear and the cells become bare just like those of distal or collecting tubules (Fig. 265). The lining cells of the very dilated "cystic" tubules

finally become so much flattened that they simulate the squamous epithelial cells lining the thin segment of the loop of Henle (Fig. 266).

While this is happening, granules appear in the cellular cytoplasm. These vary in size from under 0.2 μ to over 2 μ in diameter. Most of these granules are rounded in shape (Fig. 267), but some are ring-shaped (Fig. 267 and 268) and others are more or less oval (Fig. 268) and some have various abnormal vacuolated shapes (Fig. 269). The density of the granules varies significantly from one to another (Fig. 270). From their appearance, these granules gave the impression as if they had resulted from the deposition of an insoluble material into the cellular cytoplasm after it had been incorporated excessively from the tubular lumen in a soluble form, forming the cytoplasmic vacuoles followed by the absorption of the water to the blood stream leaving behind a deposit into the cytoplasm. A strong evidence in favour of this suggestion is the fact that the granules usually appear initially ring-shaped, in close contact with a mitochondrion which is apparently actively responsible for the absorption of the fluid from the vacuole leaving the deposit at its rim peripherally (Fig. 271).

An important finding, hitherto undescribed, concerning the role played by the microvilli in the process of absorption has been demonstrated in the tubules that have partially lost their microvilli. Apparently two adjacent microvilli come in contact with each other at their luminal ends enclosing a droplet of tubular fluid, then by a process of progressive sealing off towards the lumen and opening up towards the cell, the droplet moves into the cell, as a bolus of food moves down the gullet, and becomes incorporated into the superficial relatively clear zone of the cytoplasm as a vacuole (Fig. 272 and 273). This could be demonstrated in these pathologically

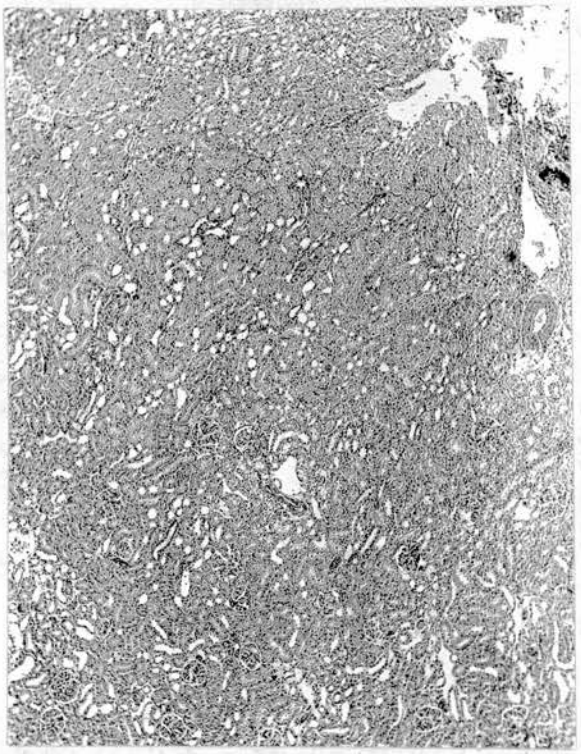


Fig. 249. A low power view of the cortex and cortico-medullary zone from a control animal. x 50

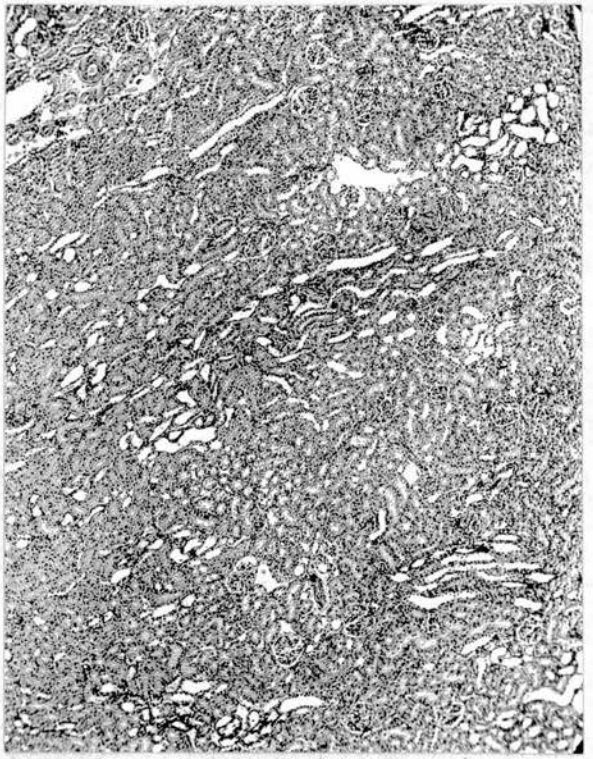


Fig. 250. A low power view of the cortex and cortico-medullary zone from a 13-day magnesium deficient rat. Note the foci of dilated tubules. x 50

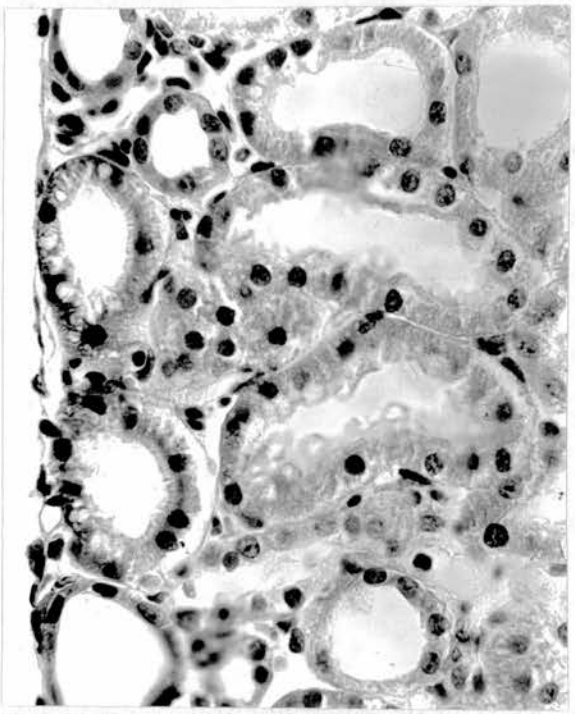


Fig. 251. A focus of dilated cortical tubules in a 13-day magnesium deficient rat. A definite brush border can be seen in some of the tubules indicating they are proximal. Note that the lining cells in some tubules are swollen and vacuolated, showing an apparent "hydropic" degeneration. x 525.

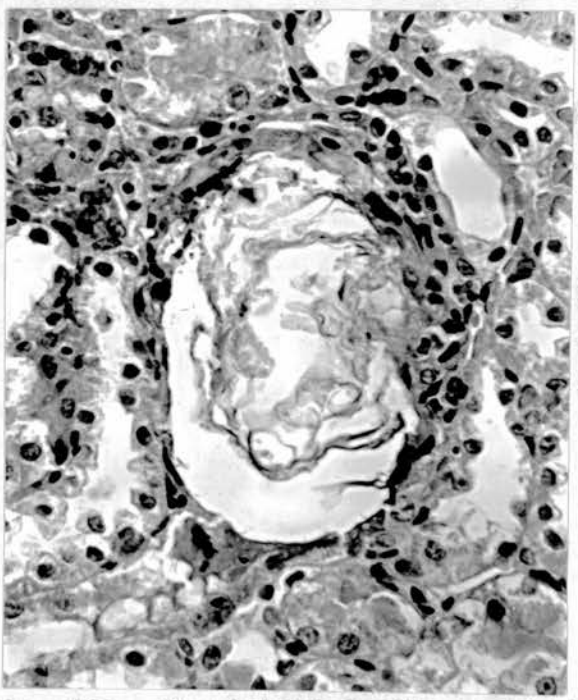


Fig. 252. The tubule in the centre is very dilated, its epithelium flattened and its lumen filled by a deposit not unlike a nidus of a calculus. From the outer zone of the medulla of a 13-day magnesium deficient rat. x 475.

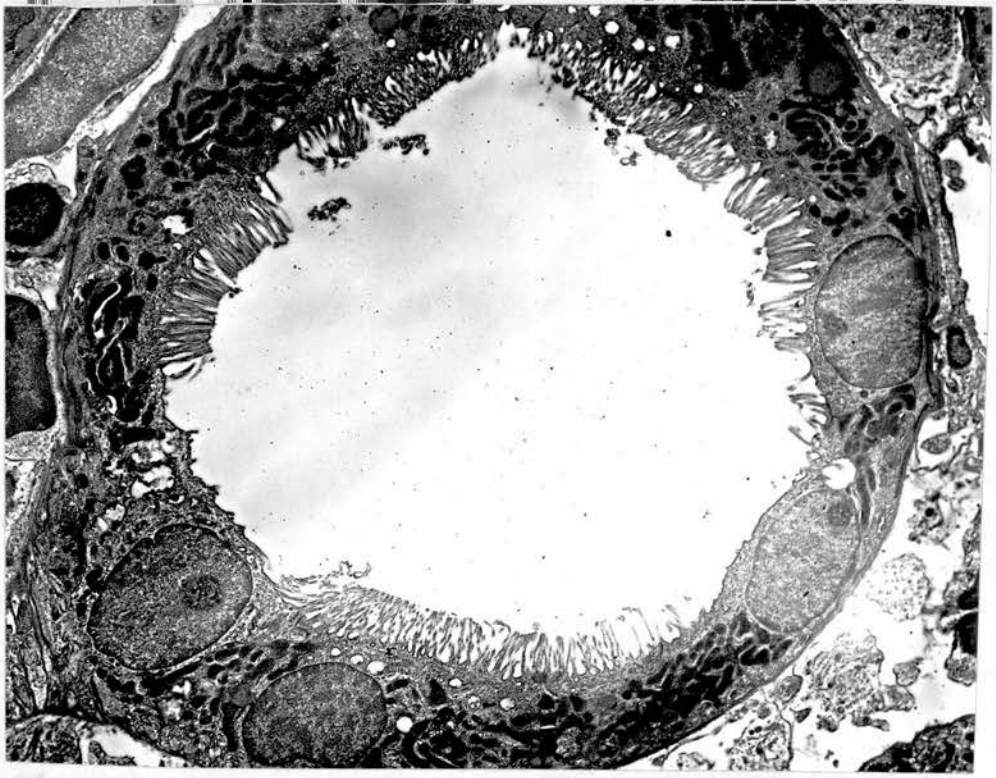


Fig. 253. Pars recta of a proximal tubule from a 3-day magnesium deficient rat. The lumen is very wide and some of the lining cells have lost their microvilli. x 1000

Fig. 254. Parts of three pars recta of proximal tubules froma 3-day magnesium deficient rat are seen. The cells have largely lost their microvilli and the cytoplasm contains clear vacuoles and a few structureless granuoles. x 2,500

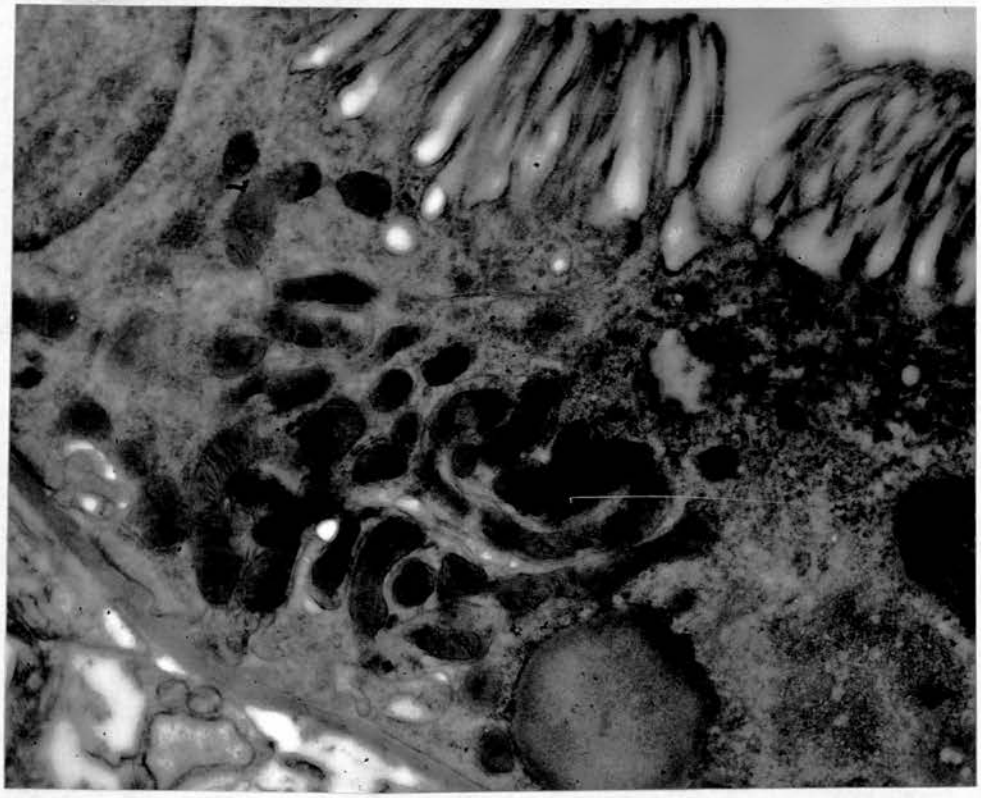


Fig. 255. Part of a cell of a pars recta of a proximal tubule from a 3-day magnesium deficient rat. A structureless granule, several times the size of the neighbouring mitochondria is seen in the basal part of the cell. x 12,000

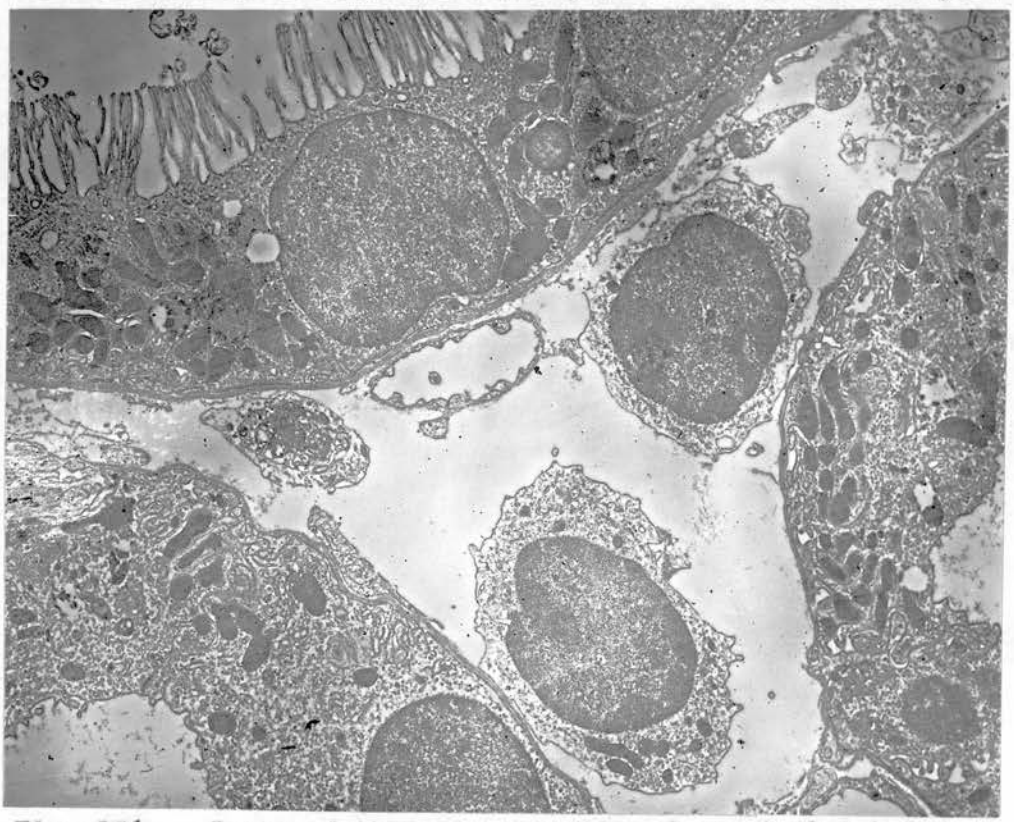


Fig. 256. Parts of three pars rectae of proximal tubules from a 3-day magnesium deficient rat. The tubule at the top has partly lost its microvilli, while the two other tubules have completely lost their brush border. x 4000

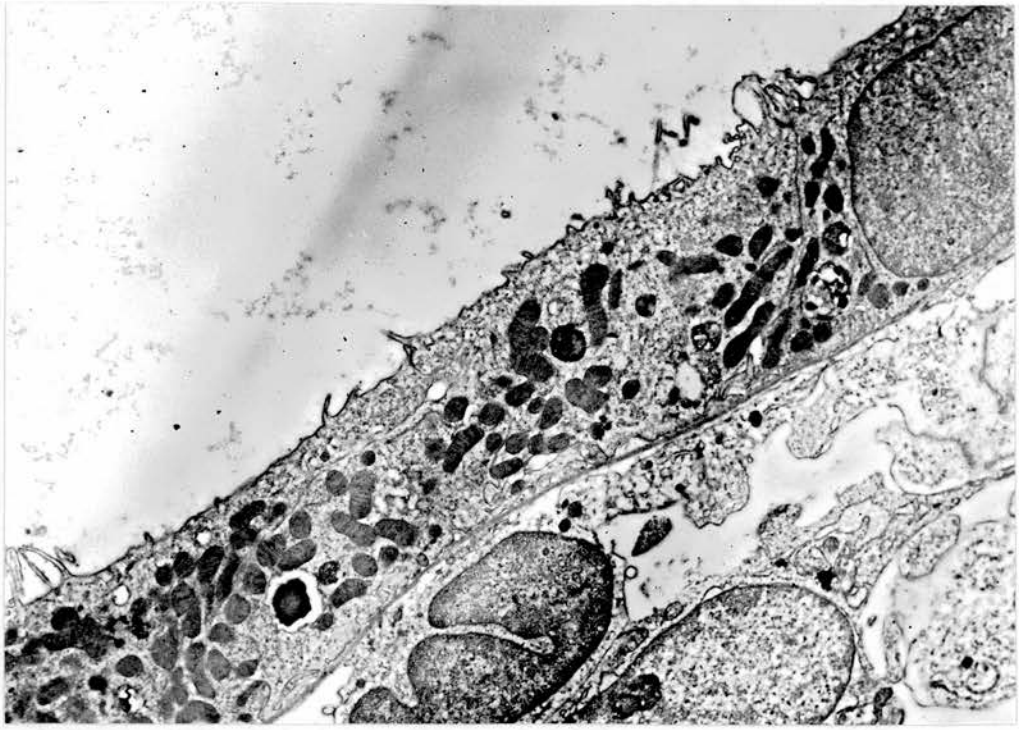


Fig. 257. Pars recta of a proximal tubule from a 3-day magnesium-deficient rat. The tubule is very dilated, the cells are flattened and have more or less completely lost their microvilli. x 4,000

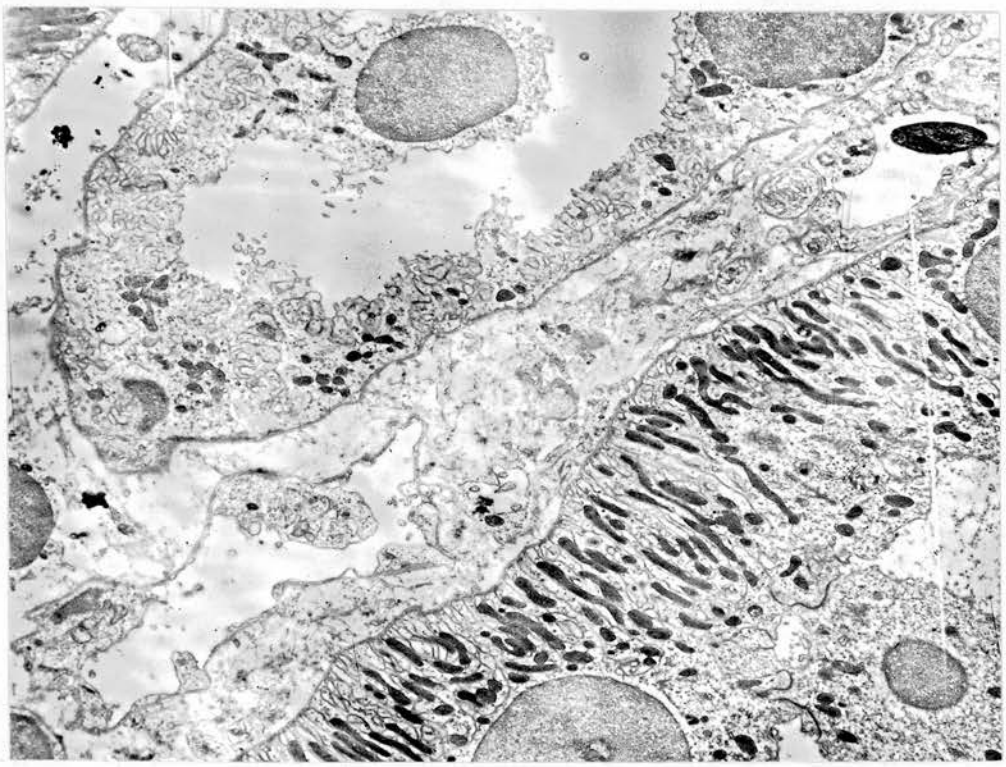


Fig. 258. A thin and a thick segment of the loop of Henle from a 13-day magnesium deficient rat, looking absolutely normal. x 4,000

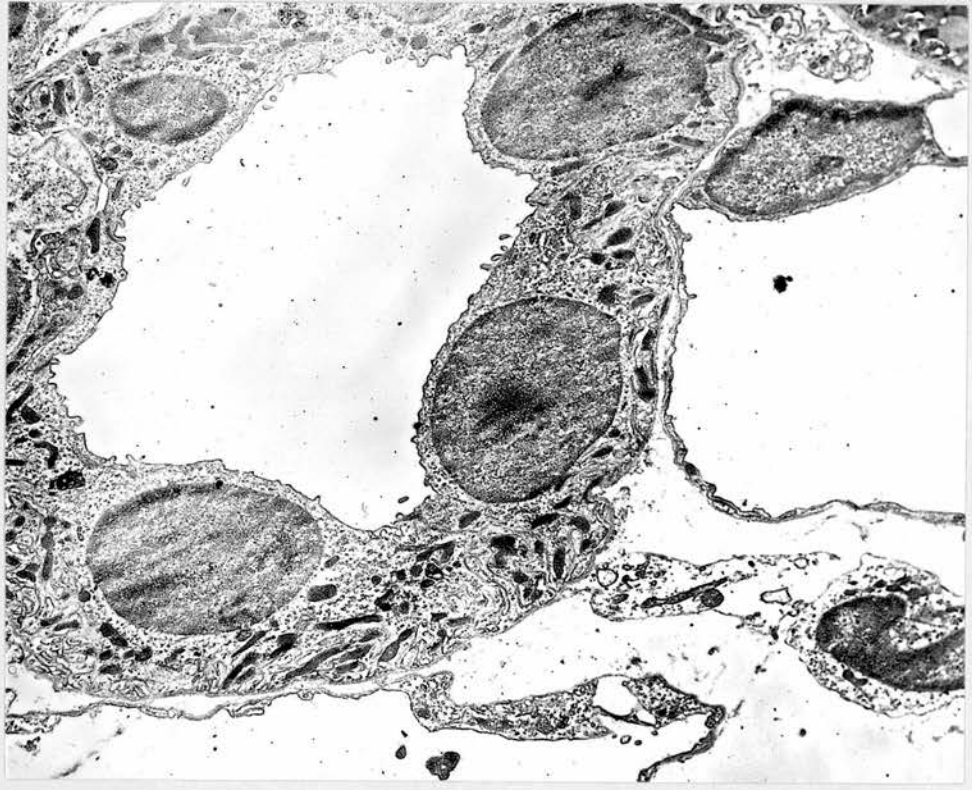


Fig. 259. A tubule in the outer zone of the medulla of a 13-day magnesium deficient rat, not conforming to the ideal description of either a proximal or distal or a collecting tubule. x 4,000

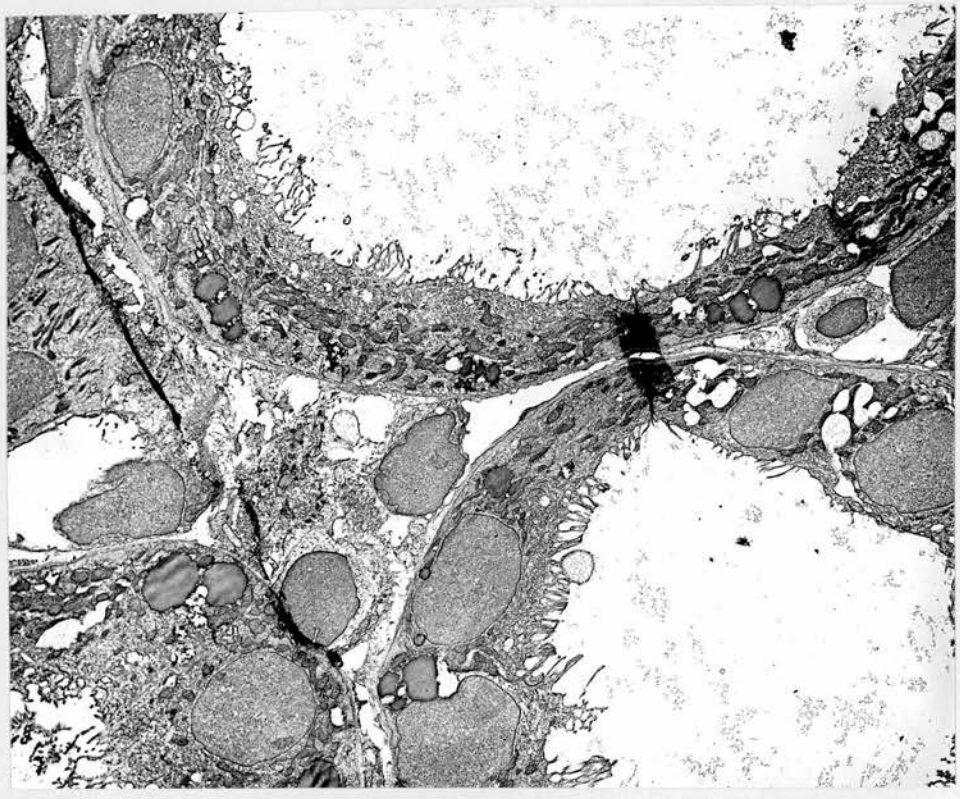


Fig. 260. Three proximal convoluted tubules from a 13-day magnesium deficient rat showing dilatation of lumen, loss of microvilli and the appearance of vacuoles and dense bodies in the cytoplasm. x 1000

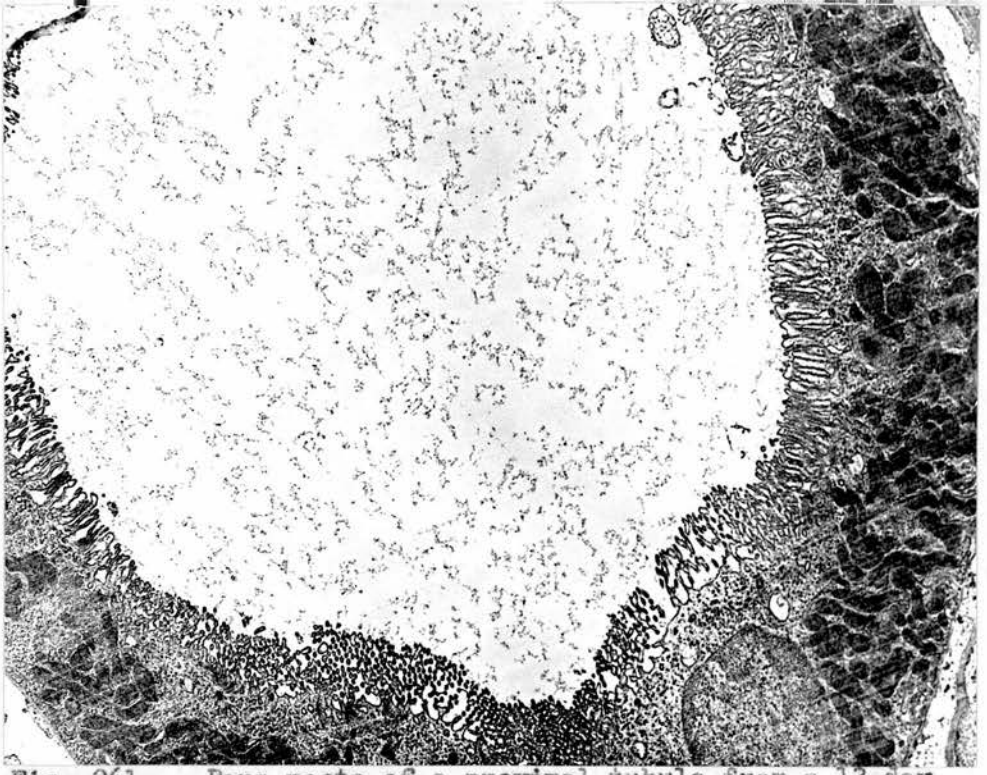


Fig. 261. Pars recta of a proximal tubule from a 13-day magnesium deficient rat only showing dilatation of the lumen; the microvilli are intact and the cells contain neither vacuoles nor granules. x 25,000

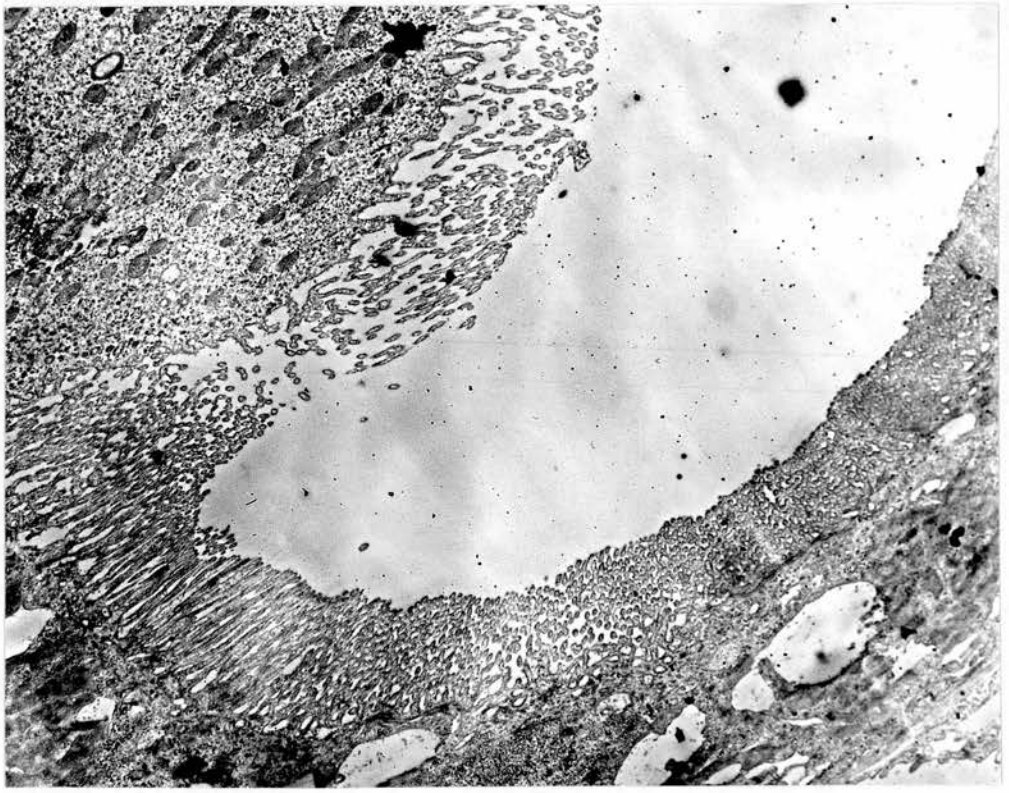


Fig. 262. Proximal convoluted tubule from a 13-day magnesium deficient rat. In addition to the dilated lumen many vacuoles are seen in the cytoplasm. The microvilli are more or less intact. x 6,000

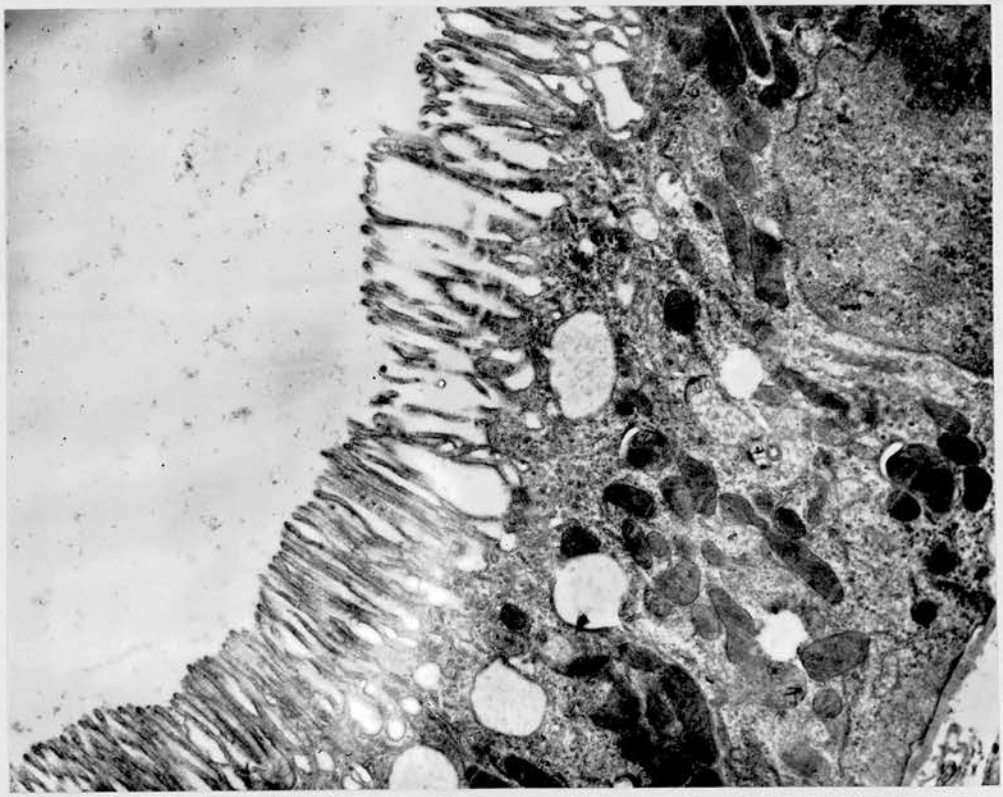


Fig. 263. Proximal convoluted tubule-cell from a 13-day magnesium-deficient rat. In addition to the large number of cytoplasmic vacuoles, the microvilli are beginning to become sparse. x 6,000

Fig. 264. Parts of three proximal convoluted tubules from a 13-day magnesium deficient rat. The microvilli have largely disappeared and the cytoplasm is full of many clear vacuoles and a few structureless granules. x 2,500

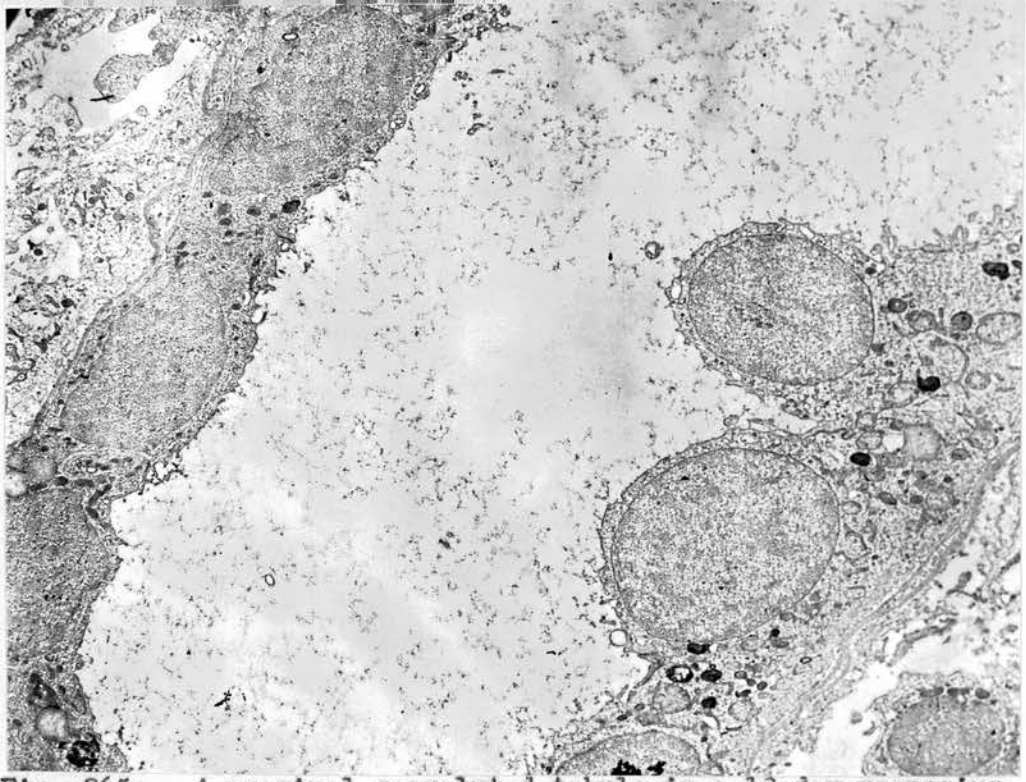


Fig. 265. A proximal convoluted tubule in a 13-day magnesium deficient rat. The dilated lumen and the absence of microvilli makes this tubule not unlike a collecting tubule. x 2,500.

Fig. 266. An extremely dilated proximal convoluted tubule from a 13-day magnesium deficient rat. The marked flattening of the cells and the absence of microvilli makes them similar to the flat squamous epithelial cells lining the thin segment. x 2,500

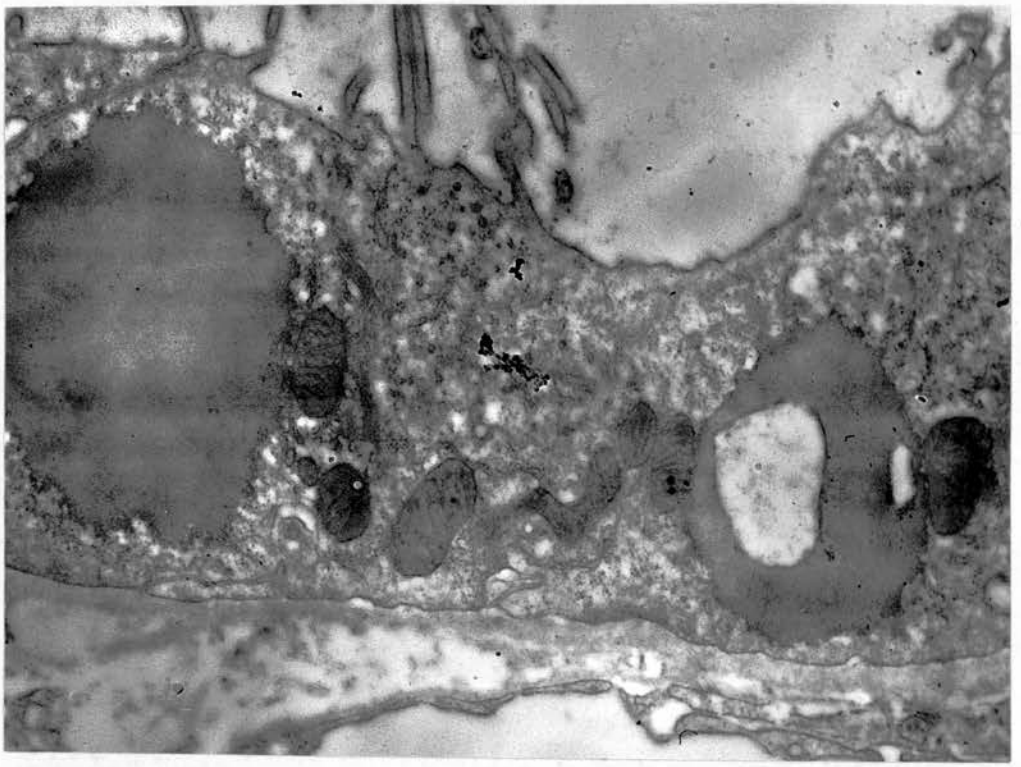


Fig. 267. Part of a proximal convoluted tubule cell from a 13-day magnesium deficient rat. A large rounded structureless granuole is seen on the left and a smaller ring-shaped granuole on the right. x 15,000

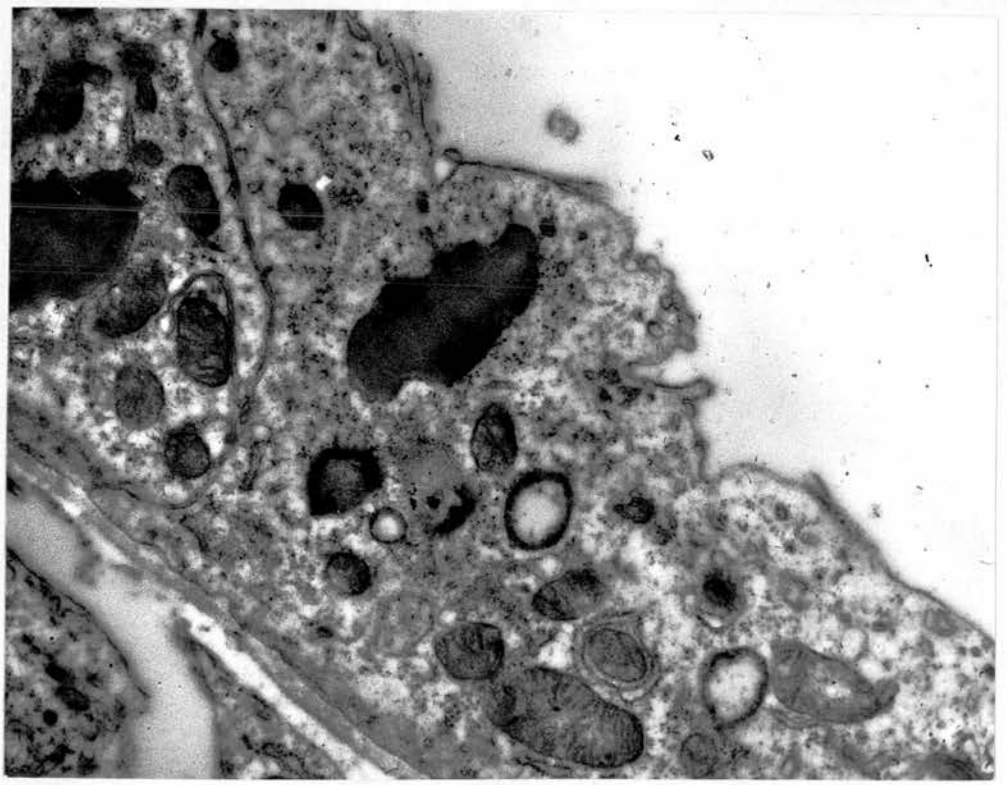


Fig. 268. Cell of a pars recta of a proximal tubule from a 13-day magnesium deficient rat. Note the oval and the ring-shaped structureless granules. x 15,000

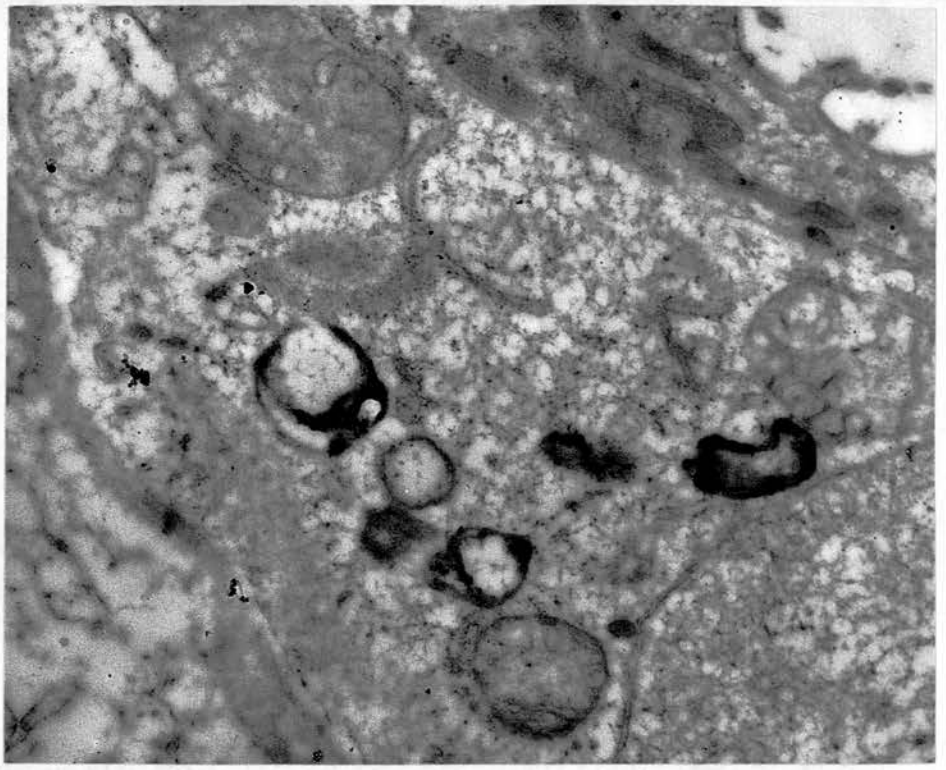


Fig. 269. Part of a proximal tubule cell from a 13-day magnesium deficient rat. Note the curiously shaped, vacuolated granules. x 24,000

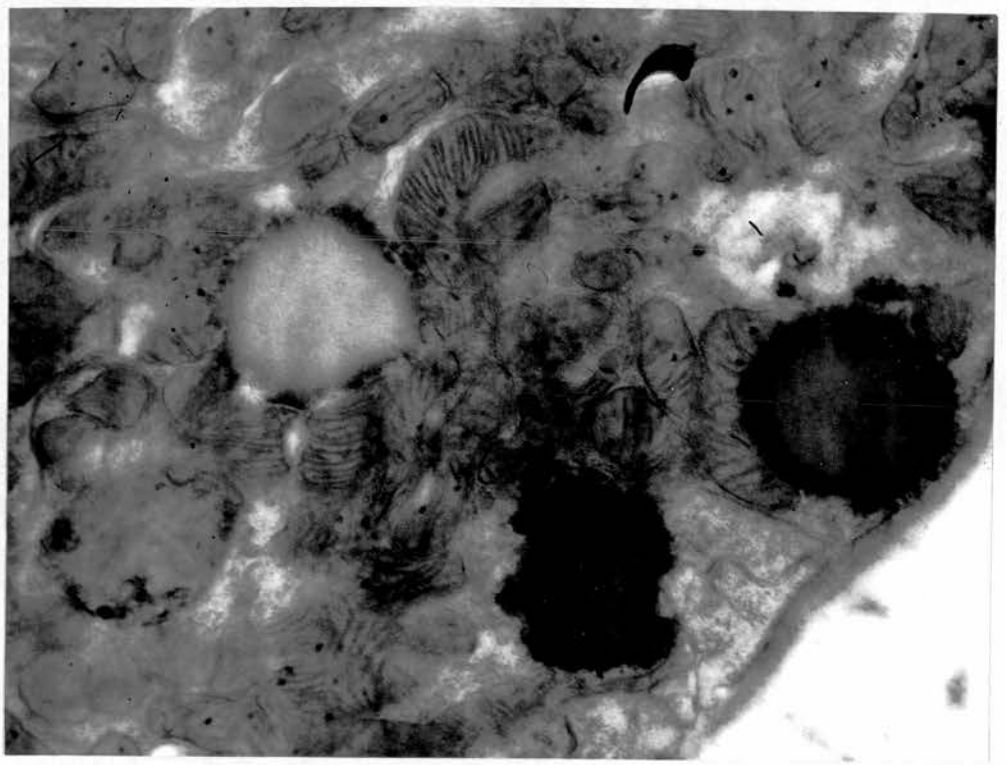


Fig. 270. Part of a proximal convoluted tubule cell from a 13-day magnesium deficient rat. Four structureless granules are seen amidst the mitochondria. The top one is very weakly osmiophilic except for its rim, the one on the left is moderately osmiophilic with several small dense dots, the two on the right, just above the basement membrane are very strongly osmiophilic. x 24,000

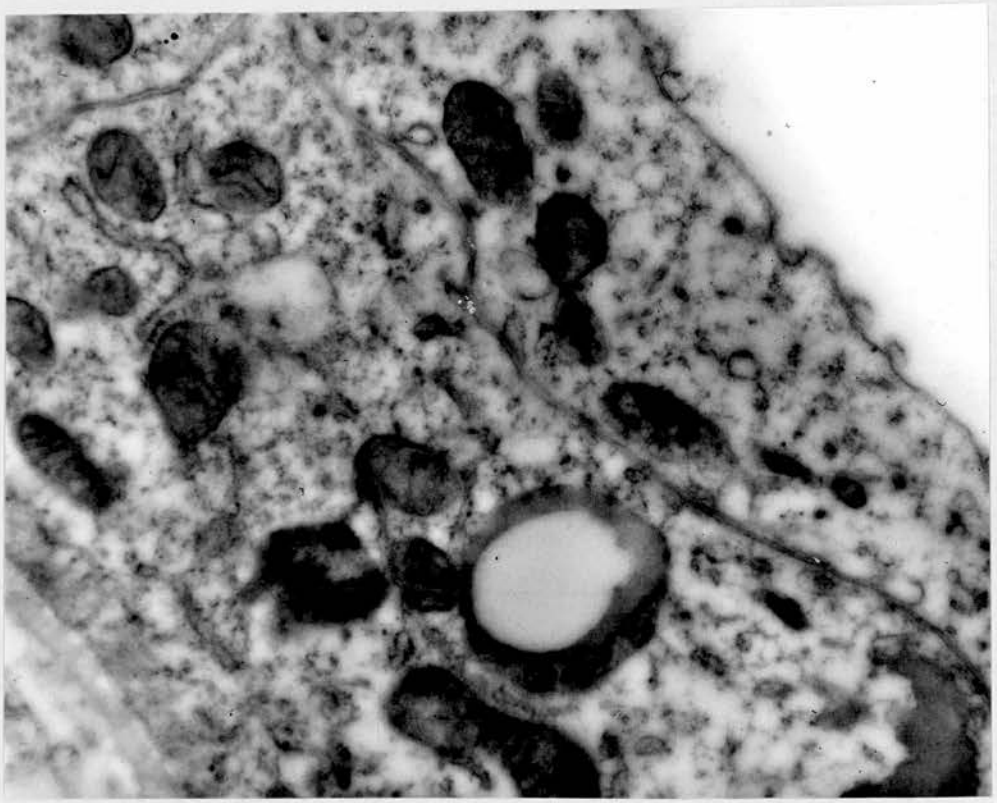


Fig. 271. Cell of pars recta of a proximal tubule from a 13-day magnesium-deficient rat. In the middle a vacuole is seen with a crescentic mitochondria closely applied to its surface. A structureless deposit is seen at the periphery of the vacuole. A crescentic granule is seen in the bottom right. x 24,000

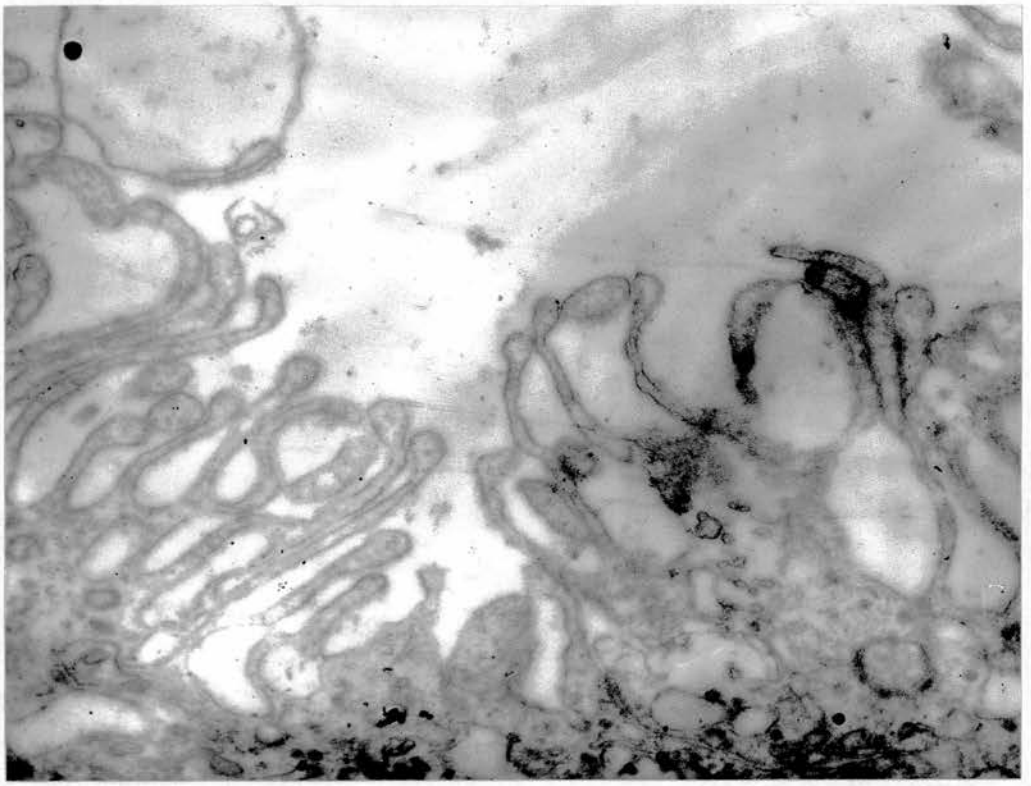


Fig. 272. The brush border of a proximal tubule cell from a 13-day magnesium-deficient rat. Note how the neighbouring microvilli enclose droplets of tubular fluid. x 30,000

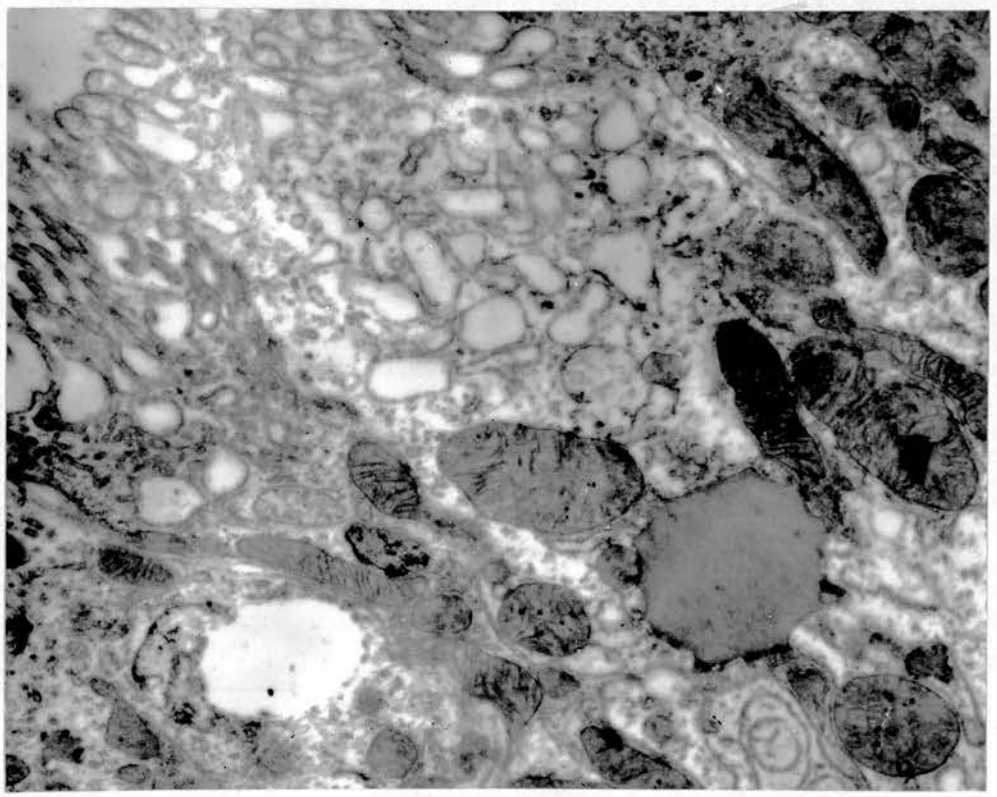


Fig. 273. Proximal convoluted tubule cell from a 13-day magnesium deficient rat. The droplets of tubular fluid enclosed between neighbouring microvilli have moved down and appear as vacuoles in the superficial part of the cell.
x 19,500

altered tubules, with scarce microvilli while it is apparently impossible to demonstrate in the normal tubules with very crowded microvilli. This finding also gives support to the suggestion just made about the pathogenesis of the cytoplasmic vacuoles and granules found in these cells.

Important negative findings to be stressed are the normal appearances of the mitochondria, the normal thickness and appearance of the basement membrane and the absence of any demonstrable obstruction in the dilated tubules or in more distal sites.

Discussion.

The changes described in the kidney in experimental depletion of magnesium in animals consisted of a progressive increase in the calcium content of that organ (38) corresponding to the calcification of the inner cortical zone observed histologically. This lesion was found almost invariably in later phases of magnesium deficiency and was considered to precede (13) or to follow (30) more extensive morphological damage of tubular epithelium. Conventional histological techniques were unable to reveal any alteration in renal cells directly dependent on the induced electrolyte imbalance and leading to necrosis and calcification. Histochemical techniques (19) were claimed to demonstrate mitochondrial swelling and alteration in the enzymatic activity in the cells of the proximal tubule in dietary deficiency of magnesium. No study of magnesium deficiency nephropathy by the electron microscope has been previously described.

In the study reported in this thesis, electron microscopy has definitely shown that the lesion of magnesium depletion is localised at least initially, to the proximal tubules, and begins in the pars recta then

extends to the pars convoluta. No swelling or morphological change was found in the mitochondria. The lesion consists of dilatation of the tubule, initial swelling and vacuolisation of the cells, followed by the gradual disappearance of the microvilli, the deposition of dense granules in the cytoplasm and finally the extreme flattening out of the cells lining the very dilated "cystic" tubules.

The vacuoles seen in the cytoplasm are apparently hydropic and not lipoid droplets as described by Hess et al (19); the latter would have been very densely osmiophilic.

The exact nature of the dense granuoles could not be ascertained in this investigation, although various evidences point to them as being calcium granules. The von Kossa stain (which however is not very specific for calcium) did not give conclusive results. However, the fact that the chemical analysis of the renal tissue showed a marked rise in the calcium content in the kidneys where these granules were encountered is an indirect strong evidence that they are calcium granules. They are not the cause of the dilatation of the tubules or the direct cause for the disappearance of the microvilli, because these two changes are seen at an earlier stage than the appearance of the dense granules. Also, they do not follow cellular necrosis, because no necrosis of cells was observed. They apparently result from the deposition into the cell cytoplasm of "calcium" reabsorbed from the tubular fluid.

Calcium and magnesium metabolism seem to be very much inter-related. The largest amount of both elements is present in bone. It has been shown that the parathyroid glands influence magnesium as well as calcium metabolism, hyperparathyroidism being associated with a negative magnesium as well as a

negative calcium balance (18) and prolonged administration of parathyroid extract increased the faecal excretion of magnesium (14). In animals, it has been shown that magnesium absorption from the gut varies inversely with the calcium intake and it was suggested that the two elements are absorbed by a common transport system (1). A common renal tubular reabsorptive system for calcium and magnesium has also been suggested by Berglung and Forster (5) from their work in the aglomerular teleost and by Alcock and MacIntyre (1) from experiments on the excretion of calcium in magnesium deficient rats. What seems to occur in magnesium deficiency is that the proximal tubule cells which normally reabsorb Ca^{++} and Mg^{++} by a common transport mechanism reabsorb much more Ca^{++} from the glomerular filtrate which is deficient in Mg^{++}. The excessively absorbed calcium ions are largely deposited locally in these cells, and appear as the dense granules, and do not pass to the general circulation. This would explain the high calcium content in the kidneys(and the nephrocalcinosis in late stages) in presence of a normal calcium in the extracellular fluid (evidenced by the normal serum calcium level in presence of hypomagnesia) and the normal calcium content of the intracellular fluid (evidenced by the normal calcium content in the muscles which have become depleted of magnesium).

The morphologically apparent alterations seen in the proximal tubules might thus be the direct result of either the magnesium deficiency or of the calcium excess. Magnesium ions are intracellular ions, present in all the cells, and essential in the maintenance of mitochondrial structure and function (32). The facts that the observed changes were localised to the proximal tubules and did not affect the entire nephron, that the mitochondria were entirely normal even in the altered cells and that the magnesium content

of the kidney was normal, exclude the suggestion that a deficiency in magnesium ions is the direct cause for the lesions. It seems that it is that segment of the nephron which is reabsorbing calcium excessively in magnesium depletion, in the interest of the general metabolism of the body, which succumbs to the toxic effect of a very high level of calcium ions passing through, loses its reabsorbing microvilli and degenerate.

The studies presented in this and in the previous chapter raise a very interesting point. In pure depletion of potassium, no lesions were detected in the proximal tubules and the earliest abnormality in renal function was the excretion of a large volume of dilute urine. In pure depletion of magnesium, lesions were localised to the proximal tubules, and no change in the volume of urine was noticed. In the reported human cases of what has been termed chronic depletion of potassium, the kidneys frequently showed lesions in the proximal tubules and the patients nearly always passed a large volume of dilute urine. When these case reports have been restudied (as reviewed in the previous chapter) it was apparent that not a single case could be considered as a case of pure ptoassium depletion. One group of patients suffered from excessive and prolonged losses of gastrointestinal fluids and such losses would produce depletion of both the body potassium and magnesium. The other group were patients with primary aldosteronism. This condition is well known to produce an excessive loss of potassium in the urine. But it also produces (though not generally appreciated) an excessive loss of magnesium in the urine and faeces as has been demonstrated in the two cases where magnesium balance studies were carried out (28,29) and as has been definitely proved in experimental studies in the rat by Hanna and MacIntyre (17). Most of these reported cases,

therefore had negative magnesium balance in addition to the negative potassium balance, though no effort was made to demonstrate it because the significance of body depletion of magnesium was not realised. The lesions in the proximal tubules in the reported human cases, can thus be very easily explained to be the result of magnesium depletion while the passage of a large volume of urine to be due to potassium depletion. This hypothesis can only be definitely proved if, when future cases of primary aldosteronism or chronic losses of gastrointestinal fluids are encountered, an accurate potassium and magnesium (and preferably also calcium) balance study is carried out, a renal biopsy is taken during the deficiency state and examined by the electron, in addition to the light microscope, then careful repletion of one element only is done and a repeat renal biopsy and renal function studies are performed, then the other element is repleted and the same procedures again repeated.

The demonstration that magnesium depletion results in remote sequelae in the kidney, eventually leading to the unalterable serious condition of nephrocalcinosis should urge clinicians to be much more aware of the possibility of an existing magnesium loss in the patients under their care, should stimulate them to remove the cause of this magnesium loss as quickly as possible and should indicate to them the administration of sufficient supplements of magnesium until the removal of the cause is feasible, in order to avert this dangerous complication in the kidney.

REFERENCES.

1. Alcock, N. and MacIntyre, I.
Inter-relation of calcium and magnesium absorption.
Biochem. J., 76: 19, 1960.

2. Alcock, N., MacIntyre, I. and Radde, I.
The determination of magnesium in biological fluids and tissues by flame spectrophotometry.
J. Clin. Path., 13: 506, 1960.

3. Barron, G.P., Brown, S.O. and Pearson, P.B.
Histological manifestations of a magnesium deficiency in the rat and rabbit.
Proc. soc. Exper. Biol. & Med., 70: 220, 1949.

4. Blaxter, K.L., Rook, J.A.F. and MacDonald, A.M.
Experimental magnesium deficiency in calves.
I. Clinical and pathological observations.
J. Comp. Path. & Therap., 64: 157, 1954.

5. Berglund, F. and Forster, R.P.
Renal tubular transport of inorganic divalent ions by the aglomerular marine teleost Laphins Americanus.
J. Gen. Physiol., 41: 429, 1958.

6. Brookfield, R.W.
Magnesium deficiency in the rat.
Brit. Med. J., 1: 848, 1934.

7. Card, W.I. and Marks, I.N.
A case of magnesium deficiency IN water and electrolyte metabolism in relation to age and sex. Ciba Foundation Colloquia on Ageing 4: 301, 1958.

8. Cramer, W.
Experimental production of kidney lesions by diet.
Lancet 2: 174, 1932.

9. Dawson, J.B. and Heaton, F.W.
The determination of magnesium in biological materials by atomic absorption spectrophotometry.
Biochem. J.,80: 99, 1961.

10. Fletcher, R.F., Henly, A.A., Sammons, H.G. and Squire, J.R.
A case of magnesium deficiency following massive intestinal resection.
Lancet 1: 522, 1960.

11. Forbes, R.M., Mitchell, H.H. and Cooper, A.R.
Further studies on the gross composition and mineral element in the adult human body.
J. biol. Chem., 223: 969, 1956.

12. Fourman, P.
Magnesium metabolism in man.
Sci. Basis Med. Ann. Rev., 17: 270, 1961.

13. Greenberg, D.M., Lucia, S.P. and Tufts, E.V.
The effect of magnesium deprivation on renal function.
Amer. J. Physiol., 121: 424, 1938.

14. Greenwald, I. and Gross, J.
The effect of the administration of a potent parathyroid extract upon the excretion of nitrogen, phosphorous, calcium and magnesium with some remarks on the solubility of calcium phosphate in serum and of thepathogenesis of tetany.
J. biol. Chem., 66: 217, 1925.

15. Hanna, S.
Plasma magnesium in health and disease.
J. clin. Path., 14: 410, 1961.

16. Hanna, S., Harrison, M., MacIntyre, I and Fraser, R.
The syndrome of magnesium deficiency in man.
Lancet 2: 172, 1960.

17. Hanna, S. and MacIntyre, I.
The influence of aldosterone on magnesium metabolism.
Lancet 2: 348, 1960.

18. Hanna, S., North, K.A.K., MacIntyre, I. and Fraser, R.
Magnesium metabolism in parathyroid disease.
Brit. Med. J., 2: 1253, 1961.

19. Hess, R., MacIntyre, I., Alcock, N. and Pearson, A.G.E.
Histochemical changes in rat kidney in magnesium deprivation.
Brit. J. Exper. Path., 40: 80, 1959.

20. Irving, E.A. and Watts, P.S.
Estimation of calcium and magnesium in blood serum by cathode ray polarograph.
Biochem. J., 79: 429, 1961.

21. Kruse, H.D., Orent, E.R. and McCollum, E.V.
Studies on magnesium deficiency in animals.
I. Symptomatology resulting from magnesium deprivation.
J. biol. Chem., 96: 519, 1932.

22. Kruse, H.D., Orent, E.R., and McCollum, E.V.
Studies on magnesium deficiency in animals.
III. Chemical changes in the blood following magnesium deprivation.
J. biol. Chem., 100: 603, 1933.

23. Kruse, H.D., Schmidt, M.M. and McCollum, E.V.
Studies on magnesium deficiency in animals.
IV. Reaction to galvanic stimuli following magnesium deprivation.
Amer. J. Physiol., 105: 635, 1933.

24. Lavollay, J.
Observations sur les accidents presentes par les jeunes rats carences en magnesium.
C.R. Soc. Biol., Paris, III: 545, 1932.

25. Lowenhaupt, E., Schulman, M.P. and Greenberg, D.M.
Basic histologic lesions of magnesium deficiency in the rat.
Arch. Path., 49: 427, 1950.

26. McCollum, E.V. and Orent, E.R.
Effects on the rat of deprivation of magnesium.
J. Biol. Chem., 92: XXX, 1931.

27. MacIntyre, I. and Davidsson, D.
The production of secondary potassium depletion, sodium retention, nephrocalcinosis and hypercalcaemia by magnesium deficiency.
Biochem. J., 70: 456, 1958.

28. Mader, I.J. and Iseri, L.T.
Spontaneous hypopotassemia, hypomagnesemia, alkalosis and tetany: Metabolic and physiologic studies.
J. Lab. Clin. Med., 44: 895, 1954.

29. Milne, M.D., Muerhrchke, R.C. and Aird, I.
Primary aldosteronism.
Quart. J. Med., 26 (N.S.): 317, 1957.

30. Moore, L.A., Hallman, E.T. and Scholl, L.B.
Cardiovascular and other lesions in calves fed diets low in magnesium.
Arch. Path., 26: 820, 1938.

31. Osborne, T.B. and Mendel, L.B.
The inorganic elements in nutrition.
J. biol. Chem., 34: 131, 1918.

32. Raaflaub, J.,
Die Schwellung isolierter Leberzellmitochondrien und ihre physikalisch - chemische Beeinflubbarkeit.
Helv. Physiol. Pharmacol. Acta, 11: 142, 1953.

33. Randall, R.E.Jr., Rossmeisl, E.C. and Bleifer, K.H.
Magnesium depletion in man.
Ann. Inter. Med., 50: 257, 1959.

34. Schrader, G.A., Prickett, C.O. and Salmon, W.D.
Symptomatology and pathology of potassium and magnesium deficiencies in the rat.
J. Nutrition, 14: 85, 1937.

35. Snedecor, G.W.
Statistical Methods applied to experiments in agriculture and biology.
5th Ed., Iowa State College Press. Ames.

36. Suguira, K. and Benedict, S.R.
The influence of magnesium on the growth of carcinoma, sarcoma and melanoma in animals.
Amer. J. Cancer, 23: 300, 1935.

37. Sullivan, M. and Evans, V.J.
Nutritional dermatoses in the rat.
IX. Evaluation of the inter-relationship of magnesium deficiency and deficiencies of the vitamin B complex.
J. Nutrition, 27: 123, 1944.

38. Tufts, E.V. and Greenberg, D.M.
The biochemistry of magnesium deficiency.
I. Chemical changes resulting from magnesium deprivation.
J. biol. Chem., 122: 693, 1938.

39. Watchorn, E. and McCance, R.A.
Subacute magnesium deficiency in rats.
Biochem. J., 31: 1379, 1937.

40. Widdowson, E.M., McCance, R.A. and Spray, C.M.
The chemical composition of the human body.
Clin. Sci., 10: 113, 1951.

41. Vallee, B.L., Wacker, W.E.C. and Elmer, D.D.
The magnesium deficiency tetany syndrome in man.
New Englan. J. Med., 262: 155, 1960.

SUMMARY.

SUMMARY.

The technique of electron microscopy, relatively recently introduced in medical research has been utilised for the study of

a) the micro-anatomy of the normal mammalian nephron.

b) the development of the renal glomerulus.

c) the morphogenesis and pathogenesis of diabetic renal lesions.

d) the mechanisms of concentration and dilution of urine in the mammalian nephron and the site and mode of action of the antidiuretic hormone.

e) the nephropathy of potassium-depletion, and

f) the nephropathy of magnesium-depletion.

The Normal Glomerulus:

The well known ultrastructural characteristics have been confirmed in human, rat and rabbit glomeruli. In addition:

1) The ultrastructure of the glomerular arteriole has been described.

2) The ultrastructure of the juxtaglomerular apparatus has been described.

3) The presence of a filtration slit-membrane between the foot processes of the epithelial cells and of attachment belts between the glomerular endothelial cells has been confirmed.

4) Only two types of cell were identified in the glomerulus: epithelial and endothelial. Evidence against the presence of a "third" type of cell has been discussed.

Glomerulogenesis.

The renal glomerulus, in the rat, has been shown to develop in situ by proliferation and readjustment of its cells and not by the invagination of a hollow vesicle by capillary loops. No "mesangial" cells have been found.

The Diabetic Renal Lesions.

A. A study of renal biopsies from young diabetic patients, with recently recognised clinical diabetis, with no clinical or laboratory evidence of renal involvement, has shown that diffuse and focal thickening of the basement membrane of the nephron are present very soon after and possibly coincidentally with the development of the defect in glucose metabolism. This suggested that the renal involvement might be an integral part of the syndrome of diabetes mellitus and not a complication. It was suggested that the thickening of the basement membrane of the nephron, such an early lesion, in the diabetic patient, might be related to an abnormality in the metabolism of mucopolysaccharides which accompanies the known abnormality in glucose metabolism.

The lesions of diffuse and possibly of nodular glomerulosclerosis were shown to be lesions of the capillary basement membrane and not inter-capillary or intracapillary.

B. Rabbits given prednisolone parenterally developed glomerular micro-aneurysms and lesions identical in their light and electron microscopic appearances with human diabetic exudative "fibrinoid" lesions. No thickening of the basement membrane was observed. It was concluded that, in the diabetic patient, the glomerular micro-aneurysms and the exudative lesions are probably related to supprarenal cortical hyperactivity.

The Normal Renal Tubules.

The known ultrastructure has been confirmed. The vascular supply has been studied in detail and the existence of a renal rete mirabile as well as the presence of two types of capillary in the renal medulla has been described. The physiological implications of the micro-morphology of the renal tubules and capillaries have been discussed.

The Mechanisms of Urinary Concentration and Dilution.

The kidney of rats from acute hydration and acute dehydration experiments, and of rats to whom pitressin has been administered intravenously have been studied by the electron microscope. The most important change noticed after forcible hydration was an immense thickening of the basement membrane of the descending limb of the loop of Henle. This finding, taken in association with various data in the literature which were difficult to explain on the basis of the generally accepted version of the countercurrent theory, has led to the presentation of a new hypothesis. In this hypothesis it is suggested that the site of action of the antidiuretic hormone is the descending limb of the loop of Henle, rather than the distal convoluted and collecting tubules. The loop thus acts as a countercurrent multiplier only in the concentrating nephron. It is claimed that this suggested hypothesis explains all the physiological data in the literature and is more acceptable teleologically.

Potassium Depletion Nephropathy.

The literature of the nephropathy of potassium depletion in human patients and in experimental animals has been fully reviewed. Humans depleted of potassium showed an inconstant lesion in the proximal tubules, animals showed lesions in the collecting tubules. Functionally, both humans and animals depleted of potassium passed a large amount of dilute urine. An inconstant lesion in the human proximal tubules cannot explain a constant functional defect.

Electron microscopic study of rats kept on a potassium deficient diet showed very soon, a thickening of the basement membrane of the descending limb of the loop of Henle very similar to that seen in acutely hydrated animals. Only when the potassium depletion was marked and had been present

for several weeks did they show changes in the collecting tubules. No lesion was observed in proximal tubular cells.

It was concluded that potassium depletion may primarily stimulate the thirst centre and that the resulting polydypsia may be responsible for the passage of a large volume of dilute urine. The inconstantly observed lesion in the human cases of potassium depletion in the proximal tubules is not due to a depletion of potassium but to some other associated factor.

Magnesium Depletion Nephropathy.

Rats kept on a magnesium deficient diet very quickly developed focal lesions in the proximal tubules: the affected tubules became dilated, their lining epithelium vacuolated and the cytoplasm contained dense structureless granuoles, possibly calcium. These changes were explained on the basis of excessive reabsorption of Ca^{++} by that segment of the nephron which normally reabsorbs both Ca^{++} and Mg^{++}.

Since the human patients who were diagnosed as having "potassium-deficiency nephropathy" were all cases of chronic and excessive loss of gastrointestinal fluid or cases of primary aldosteronism, and since these two conditions result in a negative magnesium as well as a negative potassium balance, it was suggested that the lesions in the proximal tubules in human cases of potassium-depletion, are really the result of an associated depletion of magnesium.